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(71) Applicant *(for all designated States except US)*: **NORAK BIOSCIENCES, INC.** [US/US]; 7030 Kit Creek Rd., Morrisville, NC 27560 (US).

(72) Inventors; and
(75) Inventors/Applicants *(for US only)*: **OAKLEY, Robert, H.** [US/US]; 703 Braden Dr., Durham, NC 27713 (US). **HUDSON, Christine, C.** [US/US]; 201 Brandermill Dr., Durham, NC 27713 (US).

(74) Agents: **BAUM, Allen, R.** et al.; Burns, Doane, Swecker & Mathis, L.L.P., P.O. Box 1404, Alexandria, VA 27713 (US).

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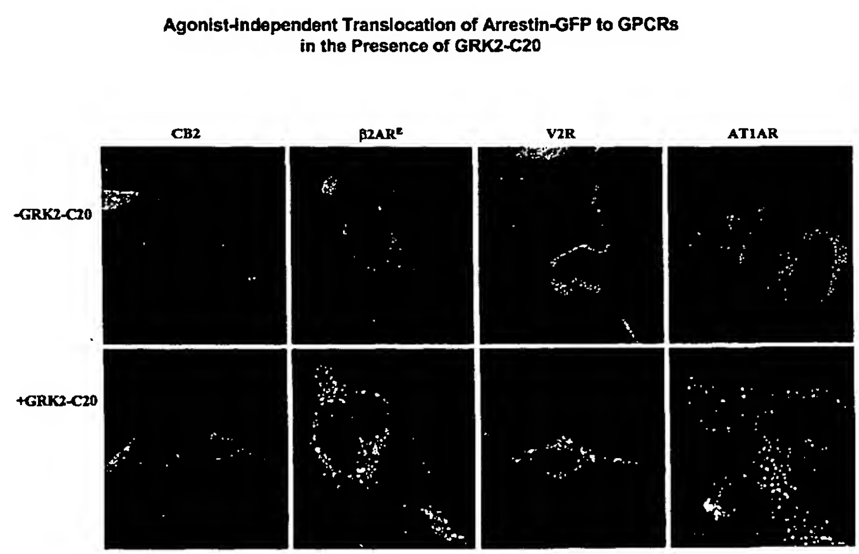
(54) Title: CONSTITUTIVELY TRANSLOCATING CELL LINE



(57) Abstract: The present invention relates to agonist-independent methods of screening for compounds that alter GPCR desensitization. Included in the present invention are cell lines containing GRKs, in which GPCRs are desensitized in the absence of agonist; the GRKs may be modified. The present invention relates to methods to determine if a GPCR is expressed at the plasma membrane, and if the GPCR has an affinity for arrestin. Modified GPCRs which have increased arrestin affinity are included in the present invention. These modified GPCRs are useful in methods to screen for compounds that alter desensitization, including both the agonist- independent methods and agonist-dependent methods described herein.

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Constitutively translocating cell line

FIELD OF THE INVENTION

[0001] The present invention relates to methods of assaying GPCR desensitization in a agonist-independent manner, host cells useful in such methods, methods of the identification of compounds that alter GPCR desensitization, the compounds identified, and their use in disease treatment.

BACKGROUND

[0002] G protein-coupled receptors (GPCRs) are cell surface proteins that translate hormone or ligand binding into intracellular signals. GPCRs are found in all animals, insects, and plants. GPCR signaling plays a pivotal role in regulating various physiological functions including phototransduction, olfaction, neurotransmission, vascular tone, cardiac output, digestion, pain, and fluid and electrolyte balance. Although they are involved in numerous physiological functions, GPCRs share a number of common structural features. They contain seven membrane domains bridged by alternating intracellular and extracellular loops and an intracellular carboxyl-terminal tail of variable length.

[0003] GPCRs have been implicated in a number of disease states, including, but not limited to: cardiac indications such as angina pectoris, essential hypertension, myocardial infarction, supraventricular and ventricular arrhythmias, congestive heart failure, atherosclerosis, renal failure, diabetes, respiratory indications such as asthma, chronic bronchitis, bronchospasm, emphysema, airway obstruction, upper respiratory indications such as rhinitis, seasonal allergies, inflammatory disease, inflammation in response to injury, rheumatoid arthritis, chronic inflammatory bowel disease, glaucoma, hypergastrinemia, gastrointestinal indications such as acid/peptic disorder, erosive esophagitis, gastrointestinal

hypersecretion, mastocytosis, gastrointestinal reflux, peptic ulcer, Zollinger-Ellison syndrome, pain, obesity, bulimia nervosa, depression, obsessive-compulsive disorder, organ malformations (for example, cardiac malformations), neurodegenerative diseases such as Parkinson's Disease and Alzheimer's Disease, multiple sclerosis, Epstein-Barr infection and cancer.

[0004] The magnitude of the physiological responses controlled by GPCRs is linked to the balance between GPCR signaling and signal termination. The signaling of GPCRs is controlled by a family of intracellular proteins called arrestins. Arrestins bind activated GPCRs, including those that have been agonist-activated and especially those that have been phosphorylated by G protein-coupled receptor kinases (GRKs).

[0005] Receptors, including GPCRs, have historically been targets for drug discovery and therapeutic agents because they bind ligands, hormones, and drugs with high specificity. Approximately fifty percent of the therapeutic drugs in use today target or interact directly with GPCRs. See e.g., Jurgen Drews, (2000) "Drug Discovery: A Historical Perspective," Science 287:1960-1964.

[0006] There is a need for accurate, easy to interpret methods of detecting G protein-coupled receptor activity and methods of assaying GPCR activity. One method, as disclosed in Barak et al., U.S. Patent Nos. 5,891,646 and 6,110,693, uses a cell expressing a GPCR and a conjugate of an arrestin and a detectable molecule, the contents of which are incorporated by reference in their entirety.

[0007] Although only several hundred human GPCRs are known, it is estimated that upwards of a thousand GPCRs exist in the human genome. Of these known GPCRs, many are orphan receptors that have yet to be associated with a ligand.

[0008] The majority of the existing methods for identifying GPCR antagonists are dependent on the presence of agonist. Assays for

identifying compounds that prevent the activation of GPCRs typically require that the GPCR is first activated in order to identify interfering compounds. For receptors with known agonists, these agonists are currently used to activate these receptors. However, many GPCRs are orphan receptors with no known ligand or agonist.

[0009] The agonist-dependence of GPCR assays continues to be a problem because antagonist discovery for orphan receptors is typically dependent on the prior discovery of agonist or ligand. Agonist-independent methods to screen for compounds that alter GPCR desensitization will (1) eliminate the step of agonist-addition in screening methods, and (2) enable identification of compounds that alter the desensitization of orphan receptors. Agonist-independent methods will eliminate the step of identifying an agonist of an orphan receptor prior to screening for compounds that alter desensitization of the orphan receptor.

SUMMARY

[0010] The present invention relates to methods of identifying compounds which alter GPCR internalization.

[0011] A first aspect of the present invention is a method of identifying a compound which alters GPCR internalization, including: (a) providing a cell including a GPCR, an arrestin, and a modified GRK, wherein said GPCR is at least partially internalized in an agonist-independent manner upon expression of said GRK; (b) exposing said cell to the compound(s); (c) determining the cellular distribution of the GPCR, arrestin, or modified GRK; and (d) monitoring a difference between (1) the distribution of the GPCR, arrestin, or modified GRK in the cell in the presence of the compound(s) and (2) the distribution of the GPCR, arrestin, or modified GRK in the cell in the absence of the compound(s). An agonist may not be provided in the above method. In the method, a difference between (1) and (2) of step (d) may indicate modulation of GPCR internalization.

[0012] The GRK may be over-expressed, its expression may be inducible, and it may include a CAAX motif. The GRK may be GRK1, GRK2, GRK3, GRK4, GRK5, GRK6, or a biologically active fragment thereof.

[0013] The GPCR may be modified to have enhanced phosphorylation by a GRK. The GPCR may be β_2AR(Y326A), a GPCR listed in Figure 1, an orphan GPCR, a modified GPCR, a taste receptor, a Class A GPCR, a Class B GPCR, a mutant GPCR, or a biologically active fragment thereof.

[0014] The arrestin may be visual arrestin, cone arrestin, β-arrestin 1, β-arrestin-2, or a biologically active fragment thereof.

[0015] The GPCR, GRK, or arrestin may be detectably labeled. A molecule involved in desensitization may be detectably labeled, or a molecule that interacts with a molecule involved in desensitization may be detectably labeled.

[0016] In a further aspect, the present invention relates to a method of identifying a compound that alters GPCR phosphorylation, including: (a) providing a cell including a GPCR and a GRK; (b) exposing the cell to the compound(s); and (c) determining whether GRK phosphorylation of the GPCR is altered in the presence of the compound(s).

[0017] The cellular distribution of the GPCR or GRK may be determined. A difference may be monitored between (1) the distribution of the GPCR or GRK in the cell in the presence of the compound(s) and (2) the distribution of the GPCR or GRK in the cell in the absence of the compound(s). A difference may be correlated between (1) and (2) to the phosphorylation of the GPCR.

[0018] The GRK may not be located in the plasma membrane, indicating that GRK phosphorylation of the GPCR is altered. The phosphorylation state of the GPCR may be determined. The activity of the GRK may be determined. The ability of the GPCR to be internalized may be determined.

[0019] In an additional aspect, the present invention relates to a method

of determining if a GPCR is expressed at the plasma membrane, including: (a) providing a cell including a GPCR, an arrestin, and a GRK, wherein the arrestin is detectably labeled; (b) determining the cellular distribution of the arrestin; and (c) correlating the cellular distribution of the arrestin to the ability of the GPCR to be expressed at the plasma membrane. The arrestin may be localized in vesicles, pits endosomes, or elsewhere in the desensitization pathway.

[0020] Additionally, the present invention relates to a further method of determining if a GPCR is expressed at the plasma membrane, including: (a) providing a cell including a GPCR and a GRK, wherein the GRK is detectably labeled; (b) determining the cellular distribution of the GRK, and (c) correlating the cellular distribution of the GRK to the ability of the GPCR to be expressed at the plasma membrane. The GRK may be localized at the plasma membrane.

[0021] In a further aspect, the present invention relates to a method of analyzing the ability of a GPCR to bind arrestin, including: (a) providing a cell including a GPCR, an arrestin, and a GRK, wherein the arrestin is detectably labeled; (b) determining the cellular distribution of the arrestin; and (c) correlating the cellular distribution of the arrestin to the ability of the GPCR to bind arrestin. The arrestin or the GPCR may be localized in vesicles, pits, or endosomes.

[0022] In an additional aspect, the present invention relates to a compound identified by a method of the present invention.

[0023] In a further aspect, the present invention is related to a method of treating a disease by modulating desensitization of a GPCR in a host cell, including: (a) providing a compound identified by a method of the present invention; and (b) administering the compound to a host.

[0024] Another aspect of the invention relates to a host cell including a GPCR and a modified GRK. The GRK may be inducible or over-expressed. The host cell may further include arrestin, wherein the arrestin may be

detectably labeled. The GPCR, GRK or another molecule involved in desensitization, or a molecule that interacts with a molecule involved in desensitization may be detectably labeled.

[0025] A further aspect of the present invention relates to a method of modifying a nucleic acid encoding a GRK in which a GPCR is constitutively internalized, including: (a) providing a nucleic acid encoding a GRK; (b) mutating the nucleic acid encoding a GRK such that the encoded GRK includes a CAAX motif, wherein the modified GRK phosphorylates a GPCR in the absence of agonist; and (c) expressing the modified GRK in a cell. The nucleic acid encoding a GRK may include SEQ ID No: 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, or 34.

[0026] The present invention also relates to a kit for identifying a compound that modulates the internalization of a GPCR, including a host cell including a GPCR and a modified GRK.

[0027] In a further aspect, the present invention relates to a modified GPCR including a NPXXY motif, and a carboxyl terminal tail, wherein the carboxyl terminal tail includes a putative site of palmitoylation and one or more clusters of phosphorylation, wherein the carboxyl terminal tail includes a retained portion of a carboxyl-terminus region of a first GPCR portion fused to a portion of a carboxyl-terminus from a second GPCR, and wherein the second GPCR includes the one or more clusters of phosphorylation and further includes a second putative site of palmitoylation approximately 10 to 25 amino acid residues downstream of a second NPXXY motif. The first GPCR may be a Class A receptor. The first GPCR may be hGPR3, hGPR6, hGPR12, hSREB2, hSREB3, hGPR8, or hGPR22. The second GPCR may be a Class B receptor. The Class B receptor may be selected from the group consisting of a vasopressin V2 receptor, a neurotensin-1 receptor, a substance P receptor, and an oxytocin receptor.

[0028] The present invention relates to a nucleic acid encoding a modified GPCR. Included in the present invention are nucleic acids

selected from the group consisting of SEQ ID Nos: 36, 38, 40, 42, 44, 46, 48, 50, 52, 54, 56, 58, 60, 62, 64, 66, 68, 70, 72, 74, 76, 78, 80, 82, 84, 86, 88, and 90. Also included in the present invention are expression vectors including the nucleic acid. Host cells including the expression vector or the nucleic acid are also included.

[0029] In a further aspect, the present invention relates to a method of screening compounds for GPCR activity including the steps of: (a) providing a cell that expresses at least one modified GPCR, wherein the cell further includes arrestin conjugated to a detectable molecule; b) exposing the cell to the compound; (c) detecting location of the arrestin within the cell; (d) comparing the location of the arrestin within the cell in the presence of the compound to the location of the arrestin within the cell in the absence of the compound; and (e) correlating a difference between (1) the location of the arrestin within the cell in the presence of the compound and (2) the location of the arrestin within the cell in the absence of the compound. The arrestin may be detected in endosomes, endocytic vesicles, or pits.

[0030] A further aspect of the present invention is a kit for identifying a molecule that modulates the activity of a GPCR, including a cell that expresses at least one modified GPCR, wherein the cell further includes a molecule involved in desensitization conjugated to a detectable molecule.

BRIEF DESCRIPTION OF DRAWINGS

[0031] The objects and advantages of the invention will be understood by reading the following detailed description in conjunction with the drawings in which:

[0032] Figure 1 is a list of GPCRs that may be used with the present invention.

[0033] Figure 2 is a list of GRKs that may be used with the present invention. Amino acid and nucleic acid sequences of certain GRKs are shown. The amino acid and nucleic acid sequences of GRK2-C20, a

modified GRK, are shown.

[0034] Figure 3 is a list of GPCRs that have been modified to have enhanced affinity for arrestin. The amino acid and nucleic acid sequences are shown.

[0035] Figure 4 illustrates the agonist-independent translocation of arrestin-GFP to GPCRs in the presence of GRK2-C20.

[0036] Figure 5 illustrates the agonist-independent translocation of arrestin-GFP to GPCRs in the presence of GRK2-C20.

[0037] Figure 6 illustrates the agonist-independent translocation of arrestin-GFP to GPCRs in the presence of GRK2-C20.

[0038] Figure 7 illustrates the agonist-independent translocation of arrestin-GFP to GPCRs in the presence of GRK2-C20.

DETAILED DESCRIPTION

[0039] In accordance with the present invention there may be employed conventional molecular biology, microbiology, immunology, and recombinant DNA techniques within the skill of the art. Such techniques are explained fully in the literature. See, e.g., Sambrook et al, "Molecular Cloning: A Laboratory Manual" (3rd edition, 2001); "Current Protocols in Molecular Biology" Volumes I-IV [Ausubel, R. M., ed. (2002 and updated bimonthly)]; "Cell Biology: A Laboratory Handbook" Volumes I-III [J. E. Celis, ed. (1994)]; "Current Protocols in Immunology" Volumes I-IV [Coligan, J. E., ed. (2002 and updated bimonthly)]; "Oligonucleotide Synthesis" (M.J. Gait ed. 1984); "Nucleic Acid Hybridization" [B.D. Hames & S.J. Higgins eds. (1985)]; "Transcription And Translation" [B.D. Hames & S.J. Higgins, eds. (1984)]; "Culture of Animal Cells, 4th edition" [R.I. Freshney, ed. (2000)]; "Immobilized Cells And Enzymes" [IRL Press, (1986)]; B. Perbal, "A Practical Guide To Molecular Cloning" (1988); *Using Antibodies: A Laboratory Manual: Portable Protocol No. I*, Harlow, Ed and Lane, David (Cold Spring Harbor Press, 1998); *Using Antibodies: A Laboratory Manual,*

Harlow, Ed and Lane, David (Cold Spring Harbor Press, 1999); "G Protein-Coupled Receptors" [T. Haga, et al., eds. (1999)].

[0040] Unless otherwise stated, the following terms used in the specification and claims have the meanings given below:

[0041] A "replicon" is any genetic element (e.g., plasmid, chromosome, virus) that functions as an autonomous unit of DNA replication *in vivo*; i.e., capable of replication under its own control.

[0042] A "vector" is a replicon, such as plasmid, phage or cosmid, to which another DNA segment may be attached so as to bring about the replication of the attached segment.

[0043] A "DNA molecule" refers to the polymeric form of deoxyribonucleotides (adenine, guanine, thymine, or cytosine) in either its single stranded form, or a double-stranded helix. This term refers only to the primary and secondary structure of the molecule, and does not limit it to any particular tertiary forms. Thus, this term includes double-stranded DNA found, *inter alia*, in linear DNA molecules (e.g., restriction fragments), viruses, plasmids, and chromosomes. In discussing the structure of particular double-stranded DNA molecules, sequences may be described herein according to the normal convention of giving only the sequence in the 5' to 3' direction along the nontranscribed strand of DNA (i.e., the strand having a sequence homologous to the mRNA).

[0044] An "origin of replication" refers to those DNA sequences that participate in the initiation of DNA synthesis.

[0045] A DNA "coding sequence" is a double-stranded DNA sequence which is transcribed and translated into a polypeptide *in vivo* when placed under the control of appropriate regulatory sequences. The boundaries of the coding sequence are determined by a start codon at the 5' (amino) terminus and a translation stop codon at the 3' (carboxyl) terminus. A coding sequence can include, but is not limited to, prokaryotic sequences, cDNA from eukaryotic mRNA, genomic DNA sequences from eukaryotic

(e.g., mammalian) DNA, and even synthetic DNA sequences. A polyadenylation signal and transcription termination sequence will usually be located 3' to the coding sequence.

[0046] Transcriptional and translational control sequences are DNA regulatory sequences, such as promoters, enhancers, polyadenylation signals, terminators, and the like, that provide for the expression of a coding sequence in a host cell.

[0047] The expression of a coding sequence in a host cell may be inducible. By inducible, it is meant that the expression can be regulated. For example, the nucleic acid may be present in the cell, but it is not expressed until a necessary signal is provided. Typically, inducible expression of a protein is controlled by a promoter that requires a necessary signal to induce transcription of the protein. However, expression may also be induced by a process or sequence that increases the number of DNA sequences of interest in the cell. Such processes or sequences include origins of replication, as well as the physical addition of DNA to a cell.

[0048] A "promoter sequence" is a DNA regulatory region capable of binding RNA polymerase in a cell and initiating transcription of a downstream (3' direction) coding sequence. For purposes of defining the present invention, the promoter sequence is bounded at its 3' terminus by the transcription initiation site and extends upstream (5' direction) to include the minimum number of bases or elements necessary to initiate transcription at levels detectable above background. Within the promoter sequence will be found a transcription initiation site (conveniently defined by mapping with nuclease S1), as well as protein binding domains (consensus sequences) responsible for the binding of RNA polymerase. Eukaryotic promoters will often, but not always, contain "TATA" boxes and "CAT" boxes. Prokaryotic promoters contain Shine-Dalgarno sequences in addition to the -10 and -35 consensus sequences.

[0049] An "expression control sequence" is a DNA sequence that

controls and regulates the transcription and translation of another DNA sequence. A coding sequence is "under the control" of transcriptional and translational control sequences in a cell when RNA polymerase transcribes the coding sequence into mRNA, which is then translated into the protein encoded by the coding sequence.

[0050] A "signal sequence" can be included before the coding sequence. This sequence encodes a signal peptide, N-terminal to the polypeptide, that communicates to the host cell to direct the polypeptide to the cell surface or secrete the polypeptide into the media, and this signal peptide is clipped off by the host cell before the protein leaves the cell. Signal sequences can be found associated with a variety of proteins native to prokaryotes and eukaryotes.

[0051] The term "oligonucleotide," as used herein in referring to the probe of the present invention, is defined as a molecule comprised of two or more ribonucleotides, preferably more than three. Its exact size will depend upon many factors which, in turn, depend upon the ultimate function and use of the oligonucleotide.

[0052] The term "primer" as used herein refers to an oligonucleotide, whether occurring naturally as in a purified restriction digest or produced synthetically, which is capable of acting as a point of initiation of synthesis when placed under conditions in which synthesis of a primer extension product, which is complementary to a nucleic acid strand, is induced, i.e., in the presence of nucleotides and an inducing agent such as a DNA polymerase and at a suitable temperature and pH. The primer may be either single-stranded or double-stranded and must be sufficiently long to prime the synthesis of the desired extension product in the presence of the inducing agent. The exact length of the primer will depend upon many factors, including temperature, source of primer and use of the method. For example, for diagnostic applications, depending on the complexity of the target sequence, the oligonucleotide primer typically contains 15-25 or more

nucleotides, although it may contain fewer nucleotides.

[0053] The primers herein are selected to be "substantially" complementary to different strands of a particular target DNA sequence. This means that the primers must be sufficiently complementary to hybridize with their respective strands. Therefore, the primer sequence need not reflect the exact sequence of the template. For example, a non-complementary nucleotide fragment may be attached to the 5' end of the primer, with the remainder of the primer sequence being complementary to the strand. Alternatively, non-complementary bases or longer sequences can be interspersed into the primer, provided that the primer sequence has sufficient complementarity with the sequence of the strand to hybridize therewith and thereby form the template for the synthesis of the extension product.

[0054] As used herein, the terms "restriction endonucleases" and "restriction enzymes" refer to bacterial enzymes, each of which cut double-stranded DNA at or near a specific nucleotide sequence.

[0055] A cell has been "transformed" by exogenous or heterologous DNA when such DNA has been introduced inside the cell. The transforming DNA may or may not be integrated (covalently linked) into chromosomal DNA making up the genome of the cell. In prokaryotes, yeast, and mammalian cells for example, the transforming DNA may be maintained on an episomal element such as a plasmid. With respect to eukaryotic cells, a stably transformed cell is one in which the transforming DNA has become integrated into a chromosome so that it is inherited by daughter cells through chromosome replication. This stability is demonstrated by the ability of the eukaryotic cell to establish cell lines or clones comprised of a population of daughter cells containing the transforming DNA. A "clone" is a population of cells derived from a single cell or common ancestor by mitosis. A "cell line" is a clone of a primary cell that is capable of stable growth *in vitro* for many generations.

[0056] Two DNA sequences are "substantially homologous" when at least about 65% (preferably at least about 80%, and most preferably at least about 90 or 95%) of the nucleotides match over the defined length of the DNA sequences. Sequences that are substantially homologous can be identified by comparing the sequences using standard software available in sequence data banks, or in a Southern hybridization experiment under, for example, stringent conditions as defined for that particular system. Defining appropriate hybridization conditions is within the skill of the art. See, e.g., Maniatis et al., supra; DNA Cloning, Vols. I & II, supra; Nucleic Acid Hybridization, supra.

[0057] It should be appreciated that also within the scope of the present invention are DNA sequences encoding the same amino acid sequence as SEQ ID NO: 1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37, 39, 41, 43, 45, 47, 49, 51, 53, 55, 57, 59, 61, 63, 65, 67, 69, 71, 73, 75, 77, 79, 81, 83, 85, 87, and 89, but also those which are degenerate to SEQ ID NO: 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36, 38, 40, 42, 44, 46, 48, 50, 52, 54, 56, 58, 60, 62, 64, 66, 68, 70, 72, 74, 76, 78, 80, 82, 84, 86, 88, and 90. By "degenerate to" is meant that a different three-letter codon is used to specify a particular amino acid.

[0058] "Arrestin" means all types of naturally occurring and engineered variants of arrestin, including, but not limited to, visual arrestin (sometimes referred to as Arrestin 1), cone arrestin (sometimes referred to as arrestin-4), β-arrestin 1 (sometimes referred to as Arrestin 2), and β-arrestin 2 (sometimes referred to as Arrestin 3).

[0059] "βARK1" is a GRK termed β-adrenergic receptor kinase 1, also called GRK2.

[0060] "βAR" is a GPCR termed a β-adrenergic receptor.

[0061] "Internalization" of a GPCR is the translocation of a GPCR from the cell surface membrane to an intracellular vesicular membrane, where it may be inaccessible to substances remaining outside the cell.

[0062] "Carboxyl-terminal tail" means the carboxyl-terminal tail of a GPCR following membrane span 7. The carboxyl-terminal tail of many GPCRs begins shortly after the conserved NPXXY motif that marks the end of the seventh transmembrane domain (i.e. what follows the NPXXY motif is the carboxyl-terminal tail of the GPCR). The carboxyl-terminal tail may be relatively long (approximately tens to hundreds of amino acids), relatively short (approximately tens of amino acids), or virtually non-existent (less than approximately ten amino acids). As used herein, "carboxyl-terminal tail" shall mean all three variants (whether relatively long, relatively short, or virtually non-existent), and may or may not contain palmitoylated cysteine residue(s).

[0063] "Class A receptors" preferably do not translocate together with arrestin proteins to endocytic vesicles or endosomes in association with arrestin-GFP in HEK-293 cells.

[0064] "Class B receptors" preferably do translocate together with arrestin proteins to endocytic vesicles or endosomes associated with arrestin-GFP in HEK-293 cells.

[0065] "DACs" mean any desensitization active compounds. Desensitization active compounds are any compounds that influence the GPCR desensitization mechanism by either stimulating or inhibiting the process. DACs may influence the GPCR desensitization pathway by acting on any cellular component of the process, as well as any cellular structure implicated in the process, including but not limited to: arrestins, GRKs, GPCRs, phosphoinositide 3-kinase, AP-2 protein, clathrin, protein phosphatases, and the like. DACs may include, but are not limited to, compounds that inhibit arrestin translocating to a GPCR, compounds that inhibit arrestin binding to a GPCR, compounds that stimulate arrestin translocating to a GPCR, compounds that stimulate arrestin binding to a GPCR, compounds that inhibit GRK phosphorylation of a GPCR, compounds that stimulate GRK phosphorylation of a GPCR, compounds that stimulate or inhibit GRK binding to a GPCR, compounds that inhibit

protein phosphatase dephosphorylation of a GPCR, compounds that stimulate protein phosphatase dephosphorylation of a GPCR, compounds that prevent GPCR internalization or recycling to the cell surface, compounds that regulate the release of arrestin from a GPCR, antagonists of a GPCR, inverse agonists and the like. DACs may inhibit or stimulate the GPCR desensitization process and may not bind to the same ligand binding site of the GPCR as traditional agonists and antagonists of the GPCR. DACs may act independently of the GPCR, i.e., they do not have high specificity for one particular GPCR or one particular type of GPCRs. DACs may bind the same site(s) as agonist or antagonist but do not desensitize the receptor (perhaps by not altering the receptor to be properly phosphorylated or bind to arrestin or any other protein). DACs may bind to allosteric sites on the receptor and inhibit or enhance desensitization.

[0066] "Detectable molecule" means any molecule capable of detection by spectroscopic, photochemical, biochemical, immunochemical, electrical, radioactive, and optical means, including but not limited to, fluorescence, phosphorescence, and bioluminescence and radioactive decay. Detectable molecules include, but are not limited to, GFP, luciferase, β-galactosidase, rhodamine-conjugated antibody, and the like. Detectable molecules include radioisotopes, epitope tags, affinity labels, enzymes, fluorescent groups, chemiluminescent groups, and the like. Detectable molecules include molecules which are directly or indirectly detected as a function of their interaction with other molecule(s).

[0067] "GFP" means Green Fluorescent Protein which refers to various naturally occurring forms of GFP which may be isolated from natural sources or genetically engineered, as well as artificially modified GFPs. GFPs are well known in the art. See, for example, U.S. Patent Nos. 5,625,048; 5,777,079; and 6,066,476. It is well understood in the art that GFP is readily interchangeable with other fluorescent proteins, isolated from natural sources or genetically engineered, including but not limited to, yellow

fluorescent proteins (YFP), red fluorescent proteins (RFP), cyan fluorescent proteins (CFP), blue fluorescent proteins, luciferin, UV excitable fluorescent proteins, or any wave-length in between. As used herein, "GFP" shall mean all fluorescent proteins known in the art.

[0068] "Unknown or Orphan Receptor" means a GPCR whose ligands are unknown.

[0069] "Downstream" means toward a carboxyl-terminus of an amino acid sequence, with respect to the amino-terminus.

[0070] "Upstream" means toward an amino-terminus of an amino acid sequence, with respect to the carboxyl-terminus.

[0071] Amino acid substitutions may also be introduced to substitute an amino acid with a particularly preferable property. For example, a Cys may be introduced a potential site in order to allow formation of disulfide bridges with another Cys. A His may be introduced as a particularly "catalytic" residue (i.e., His can act as an acid or base and is the most common amino acid in biochemical catalysis). Pro may be introduced because of its particularly planar structure, which induces β-turns in the protein's structure.

[0072] Two amino acid sequences are "substantially homologous" when at least about 70% of the amino acid residues (preferably at least about 80%, and most preferably at least about 90 or 95%) are identical, or represent conservative substitutions.

[0073] A "heterologous" region of the DNA construct is an identifiable segment of DNA within a larger DNA molecule that is not found in association with the larger molecule in nature. Thus, when the heterologous region encodes a mammalian gene, the gene will usually be flanked by DNA that does not flank the mammalian genomic DNA in the genome of the source organism. Another example of a heterologous coding sequence is a construct where the coding sequence itself is not found in nature (e.g., a cDNA where the genomic coding sequence contains introns, or synthetic sequences having codons different than the native gene). Allelic variations

or naturally-occurring mutational events do not give rise to a heterologous region of DNA as defined herein.

[0074] The phrase "pharmaceutically acceptable" refers to molecular entities and compositions that are physiologically tolerable and do not typically produce an allergic or similar untoward reaction, such as gastric upset, dizziness and the like, when administered to a human.

[0075] The phrase "therapeutically effective amount" is used herein to mean an amount sufficient to prevent, and preferably reduce some feature of pathology such as for example, elevated blood pressure, respiratory output, etc.

[0076] A DNA sequence is "operatively linked" to an expression control sequence when the expression control sequence controls and regulates the transcription and translation of that DNA sequence. The term "operatively linked" includes having an appropriate start signal (e.g., ATG) in front of the DNA sequence to be expressed and maintaining the correct reading frame to permit expression of the DNA sequence under the control of the expression control sequence and production of the desired product encoded by the DNA sequence. If a gene that one desires to insert into a recombinant DNA molecule does not contain an appropriate start signal, such a start signal can be inserted in front of the gene.

[0077] "Hybridization" means hydrogen bonding, which may be Watson-Crick, Hoogsteen or reversed Hoogsteen hydrogen bonding, between complementary nucleoside or nucleotide bases. For example, adenine (A) and thymine (T) are complementary nucleobases that pair through the formation of hydrogen bonds.

[0078] The term "standard hybridization conditions" refers to salt and temperature conditions substantially equivalent to 5 x SSC and 65 °C for both hybridization and wash. However, one skilled in the art will appreciate that such "standard hybridization conditions" are dependent on particular conditions including the concentration of sodium and magnesium in the

buffer, nucleotide sequence length and concentration, percent mismatch, percent formamide, and the like. Also important in the determination of "standard hybridization conditions" is whether the two sequences hybridizing are RNA-RNA, DNA-DNA or RNA-DNA. Such standard hybridization conditions are easily determined by one skilled in the art according to well known formulae, wherein hybridization is typically 10-20 °C below the predicted or determined Tm with washes of higher stringency, if desired.

[0079] By "animal" is meant any member of the animal kingdom including vertebrates (e.g., frogs, salamanders, chickens, or horses) and invertebrates (e.g., worms, etc.). "Animal" is also meant to include "mammals." Preferred mammals include livestock animals (e.g., ungulates, such as cattle, buffalo, horses, sheep, pigs and goats), as well as rodents (e.g., mice, hamsters, rats and guinea pigs), canines, felines, primates, lupine, camelid, cervidae, rodent, avian and ichthyes.

[0080] "Antagonist(s)" include all agents that interfere with wild-type and/or modified GPCR binding to an agonist, wild-type and/or modified GPCR desensitization, wild-type and/or modified GPCR binding arrestin, wild-type and/or modified GPCR endosomal localization, internalization, and the like, including agents that affect the wild-type and/or modified GPCRs as well as agents that affect other proteins involved in wild-type and/or modified GPCR signaling, desensitization, endosomal localization, resensitization, and the like.

[0081] "Modified GPCR" means a GPCR that has one or more modifications in the amino acid sequence of its carboxyl-terminal tail. As such, the carboxyl-terminal tail may be modified in whole or in part. These modifications in the amino acid sequence include mutations of one or more amino acids, insertion of one or more amino acids, deletion of one or more amino acids, and substitutions of one or more amino acids in which one or more amino acids are deleted and one or more amino acids are added in place of the deleted amino acids. Such modified GPCRs are described

herein, as well as in U.S.S.N. 09/993,844, which is incorporated herein by reference in their entireties.

[0082] "GPCR" means G protein-coupled receptor and includes GPCRs naturally occurring in nature, as well as GPCRs which have been modified.

[0083] "Putative site of palmitoylation" means an expected site of palmitate addition, preferably a cysteine residue. In the GPCRs used in the present invention, the putative site of palmitoylation is preferably 10 to 25, preferably 15 to 20, amino acid residues downstream of the NPXXY motif.

[0084] "Clusters of phosphorylation sites" mean clusters of amino acid residues that may be efficiently phosphorylated and thus readily function as phosphorylation sites. The clusters of amino acids occupy two out of two, two out of three, three out of three positions, three out of four positions, four out of four, four out of five positions, five out of five, and the like consecutive amino acid positions in the carboxyl terminal tail of a GPCR. These clusters of phosphorylation sites are preferably clusters of serine (S) and/or threonine (T) residues. Clusters of phosphorylation sites may be substituted, inserted, or added on to a GPCR sequence so that the resulting modified GPCR binds arrestin with sufficient affinity to recruit arrestin into endosomes.

[0085] "NPXXY motif" means a conserved amino acid motif that marks the end of the seventh transmembrane domain. The conserved amino acid motif begins most frequently with asparagine and proline followed by two unspecified amino acids and then a tyrosine. The two unspecified amino acids may vary among GPCRs but the overall NPXXY motif is conserved.

[0086] "Abnormal GPCR desensitization" and "abnormal desensitization" mean that the GPCR desensitization pathway is disrupted such that the balance between active receptor and desensitized receptor is altered with respect to wild-type conditions. Either there is more active receptor than normal or there is more desensitized receptor than wild-type conditions. Abnormal GPCR desensitization may be the result of a GPCR that is

constitutively active or constitutively desensitized, leading to an increase above normal in the signaling of that receptor or a decrease below normal in the signaling of that receptor.

[0087] "Biological sample" is intended to include tissues, cells and biological fluids isolated from a subject, as well as tissues, cells and fluids present within a subject; wherein said sample can be blood, serum, a urine sample, a fecal sample, a tumor sample, a cellular wash, an oral sample, sputum, biological fluid, a tissue extract, freshly harvested cells, or cells which have been incubated in tissue culture.

[0088] "Concurrent administration," "administration in combination," "simultaneous administration," or "administered simultaneously" mean that the compounds are administered at the same point in time or sufficiently close in time that the results observed are essentially the same as if the two or more compounds were administered at the same point in time.

[0089] "Conserved abnormality" means an abnormality in the GPCR pathway, including but not limited to, abnormalities in GPCRs, GRKs, arrestins, AP-2 protein, clathrin, protein phosphatase and the like, that may cause abnormal GPCR signaling. This abnormal GPCR signaling may contribute to a GPCR-related disease.

[0090] "Desensitized GPCR" means a GPCR that presently does not have ability to respond to agonist and activate conventional G protein signaling.

[0091] "Desensitization pathway" means any cellular component of the desensitization process, as well as any cellular structure implicated in the desensitization process and subsequent processes, including but not limited to, arrestins, GRKs, GPCRs, AP-2 protein, clathrin, protein phosphatases, and the like. In the methods of assaying of the present invention, the polypeptides may be detected, for example, in the cytoplasm, at a cell membrane, in clathrin-coated pits, in endocytic vesicles, endosomes, any stages in between, and the like.

[0092] "GPCR signaling" means GPCR induced activation of G proteins. This may result in, for example, cAMP production.

[0093] "G protein-coupled receptor kinase" (GRK) includes any kinase that has the ability to phosphorylate a GPCR. Certain GRKs which may be used in the present invention are listed in Figure 2. Splice variants, biologically active fragments, modified GRKs, and GRKs from animals and other organisms are included.

[0094] "*Homo sapiens* GPCR" means a naturally occurring GPCR in a *Homo sapiens*.

[0095] "Inverse agonist" means a compound that, upon binding to the GPCR, inhibits the basal intrinsic activity of the GPCR. An inverse agonist is a type of antagonist.

[0096] "Modified GRK" means a GRK modified such that it alters desensitization.

[0097] "Naturally occurring GPCR" means a GPCR that is present in nature.

[0098] "Odorant ligand" means a ligand compound that, upon binding to a receptor, leads to the perception of an odor including a synthetic compound and/or recombinantly produced compound including agonist and antagonist molecules.

[0099] "Odorant receptor" means a receptor protein normally found on the surface of olfactory neurons which, when activated (normally by binding an odorant ligand) leads to the perception of an odor.

[00100] The term "pharmaceutically acceptable carrier," as used herein means a pharmaceutically-acceptable material, composition or vehicle, such as a liquid or solid filler, diluent, excipient, solvent or encapsulating material, involved in carrying or transporting a chemical agent.

[00101] "Sensitized GPCR" means a GPCR that presently has ability to respond to agonist and activate conventional G protein signaling.

[00102] "Modulation" includes at least an up-regulation or down-regulation

of the expression, or an increase or decrease in activity of a protein. Modulation of a protein includes the up-regulation, down-regulation, increase or decrease in activity of a protein or compound that regulates a protein. Modulation also includes the regulation of the gene, the mRNA, or any other step in the synthesis of the protein of interest.

[00103] An "overexpressed" protein refers to a protein that is expressed at levels greater than wild-type expression levels.

[00104] "Modified GRK" means a GRK that has one or more modifications in the amino acid sequence at the C-terminus of the GRK. The modified GRK constitutively localizes to the plasma membrane. Preferably, the GRK is modified by the addition of a CAAX motif.

[00105] "CAAX" motif means a four amino acid sequence, wherein C is cysteine; A is an aliphatic amino acid; and X is the C-terminal amino acid of the protein.

[00106] A "constitutive" activity means an activity that occurs in the absence of agonist. For example, the modified GRK constitutively localizes to the plasma membrane means that the modified GRK localizes to the plasma membrane in the absence of agonist.

[00107] "GRK-C20" refers to a modified GRK which has the ability to have a geranylgeranyl group added to it. GRK2-C20 is a GRK2 modified in this manner. Preferably, the GRK-C20 contains a CAAX motif.

[00108] The present inventors developed an agonist-independent method to screen for compounds that alter GPCR desensitization. They developed cell lines in which GPCRs are desensitized in the absence of agonist. These cell lines include GRKs, which may be modified. Using these cell lines, they developed methods to screen for compounds that alter GPCR desensitization in the absence of agonist. These methods eliminate the step of agonist addition from the screening method. The elimination of this step (1) creates more efficient screening methods for compounds that alter

desensitization of GPCRs with known agonists, and (2) provides screening methods for compounds that alter desensitization of orphan GPCRs, which have no known agonist. They developed methods to determine if a GPCR is expressed at the plasma membrane, and determine if the GPCR has an affinity for arrestin; preferably these methods utilize an orphan GPCR and host cells containing a GRK, wherein the GPCR is at least partially internalized in an agonist-independent manner upon expression of the GRK, thus eliminating the need for agonist addition. They modified GPCRs to increase their affinities for arrestin. These modified GPCRs are useful in the agonist-independent methods to screen for compounds that alter desensitization.

[00109] GPCRs and desensitization

[00110] The exposure of a GPCR to agonist produces rapid attenuation of its signaling ability that involves uncoupling of the receptor from its cognate heterotrimeric G-protein. The cellular mechanism mediating agonist-specific or homologous desensitization is a two-step process in which agonist-occupied receptors are phosphorylated by a G protein-coupled receptor kinases (GRKs) and then bind an arrestin protein.

[00111] It is known that after agonists bind GPCRs, G-protein coupled receptor kinases (GRKs) phosphorylate intracellular domains of GPCRs. After phosphorylation, an arrestin protein associates with the GRK-phosphorylated receptor and uncouples the receptor from its cognate G protein. The interaction of the arrestin with the phosphorylated GPCR terminates GPCR signaling and produces a non-signaling, desensitized receptor.

[00112] The arrestin bound to the desensitized GPCR targets the GPCR to clathrin-coated pits or other cellular machinery for endocytosis (i.e., internalization) by functioning as an adaptor protein, which links the GPCR to components of the endocytic machinery, such as adaptor protein-2 (AP-2)

and clathrin. The internalized GPCRs are dephosphorylated and are recycled back to the cell surface resensitized, or are retained within the cell and degraded. The stability of the interaction of arrestin with the GPCR is one factor that dictates the rate of GPCR dephosphorylation, recycling, and resensitization. The involvement of GPCR phosphorylation and dephosphorylation in the desensitization process has been exemplified in U.S.S.N. 09/933,844, filed November 5, 2001, the disclosure of which is hereby incorporated by reference in its entirety.

[00113] Using methods described herein, the present inventors identified certain GPCRs which do not have an affinity for arrestin. They modified these GPCRs to comprise one or more sites of phosphorylation, preferably clusters of phosphorylation sites, properly positioned in their carboxyl-terminal tail. This modification allows the modified GPCR to form a stable complex with an arrestin that will internalize as a unit into endosomes. These modified GPCRs may be useful in methods of assaying GPCR activity. These modified GPCRs may be useful to identify agonists of the GPCRs. These modified GPCRs may be useful in the agonist-independent screening methods described herein.

[00114] Agonist-independent screening methods using GPCRs altered to contain a DRY motif are described in U.S.S.N. 10/054,616, which is incorporated herein by reference in its entirety. The alteration of the GPCR is included in that screening process; each GPCR to be utilized must be altered in that manner.

[00115] The present inventors developed agonist-independent screening methods using GRKs, which may be modified. These GRKs phosphorylate GPCRs in the absence of agonist. These phosphorylated GPCRs internalize in the absence of agonist. The present inventors developed agonist-independent methods of screening for antagonists of GPCR internalization utilizing these modified GRKs. These methods do not require the GPCR alterations described in U.S.S.N. 10/054,616.

[00116] Previously, certain GRKs were shown to constitutively localize in the plasma membrane. Inglese et al constructed GRK2-C20 which was constitutively isoprenylated and localized to the membranes.

[00117] The present inventors determined that cellular expression of GRKs that constitutively localize in the plasma membrane results in constitutive desensitization of GPCRs. These GRKs may be over-expressed, their expression may be inducible, the nucleic acids encoding them may be located in a vector or integrated into the genome. The present inventors constructed host cell expressing a GRK that constitutively localizes in the plasma membrane. These host cells may also express arrestin. To these host cells, they introduced a GPCR of interest. Using the GRK-containing cells, they developed methods to determine if a GPCR of interest is expressed at the plasma membrane, analyze the ability of a GPCR to bind arrestin, and detect constitutively desensitized GPCRs. They built upon these desensitization methods and developed agonist-independent methods of identifying compounds that alter GPCR desensitization. These methods are useful for the identification of compounds that alter the internalization of GPCRs, whether the GPCR agonist is known or unknown.

[00118] The present inventors also determined that increased expression of wild-type or modified GRKs increased desensitization, irrespective of whether the GRK constitutively localized in the plasma membrane.

[00119] The present invention is related to modified GPCRs, polypeptides of modified GPCRs, nucleic acid molecules that encode the modified GPCRs, vectors containing the nucleic acid molecules which encode the modified GPCRs, vectors enabling the nucleic acid construction of the modified GPCRs, and cells containing modified GPCRs. The invention further relates to assay systems using the modified GPCRs, assay systems using the cells containing modified GPCRs, compounds identified using the assay systems, methods of treatment using the compounds identified,

methods of disease diagnosis using the assay systems, and kits containing assay reagents of the present invention and cells of the present invention.

[00120] Mutations can be made in the GPCR or modified GPCR such that a particular codon is changed to a codon which codes for a different amino acid. Such a mutation is generally made by making the fewest nucleotide changes possible. A substitution mutation of this sort can be made to change an amino acid in the resulting protein in a non-conservative manner (i.e., by changing the codon from an amino acid belonging to a grouping of amino acids having a particular size or characteristic to an amino acid belonging to another grouping) or in a conservative manner (i.e., by changing the codon from an amino acid belonging to a grouping of amino acids having a particular size or characteristic to an amino acid belonging to the same grouping). Such a conservative change generally leads to less change in the structure and function of the resulting protein. A non-conservative change is more likely to alter the structure, activity or function of the resulting protein. The present invention should be considered to include sequences containing conservative changes which do not significantly alter the activity or binding characteristics of the resulting protein.

[00121] In a particular embodiment, the modified GPCRs of the present invention include GPCRs that have been modified to have one or more sites of phosphorylation, preferably clusters of phosphorylation sites, properly positioned in its carboxyl-terminal tail. These modified GPCRs recruit arrestin to endosomes within approximately 30 minutes of agonist stimulation. These modified GPCRs recruit arrestin to endosomes in the cells described herein, in which the GPCR is phosphorylated in an agonist-independent manner.

[00122] The modified GPCRs of the present invention comprise one or more sites of phosphorylation, preferably one or more clusters of phosphorylation sites, properly positioned in its carboxyl-terminal tail. The

present inventors have discovered that GPCRs containing one or more sites of phosphorylation, preferably clusters of phosphorylation sites, properly positioned in its carboxyl-terminal tail have an increased affinity for arrestin and colocalize with arrestin in endosomes upon GPCR phosphorylation, either after stimulation with agonist or in an agonist-independent manner as described herein. The present inventors have also discovered that the one or more sites of phosphorylation, preferably clusters of phosphorylation sites, must be optimally positioned within the GPCR tail for the GPCR to have an increased affinity for arrestin. Therefore, the modified GPCRs may be constructed such that the one or more sites of phosphorylation, preferably clusters of phosphorylation sites, are optimally positioned within the carboxyl-terminal tail. The portions of polypeptides, which are to be fused together to form the modified GPCR, are chosen such that the one or more sites of phosphorylation, preferably clusters of phosphorylation sites, are reliably positioned properly within the carboxyl-terminal tail. In the alternative, the location of discrete point mutations to create the modified GPCR may be chosen so that the one or more sites of phosphorylation, preferably clusters of phosphorylation sites, are properly positioned within the carboxyl-terminal tail.

[00123] The present inventors have discovered that the modified GPCRs of the present invention are useful in assays for screening compounds that may alter G protein-coupled receptor (GPCR) activity. Examples of assays in which the present invention may be used include, but are not limited to, those as described in U.S. Patent Nos. 5,891,646 and 6,110,693, the disclosures of which are hereby incorporated by reference in their entireties. Additional examples of assays in which the present invention may be used include, but are not limited to, assays using Fluorescent Resonance Energy Transfer (FRET) and assays using Bioluminescence Resonance Energy Transfer (BRET) technology as described in Angers, S., Salahpour, A., Joly, E., Hilairet, S., Chelsky, "β_2-adrenergic receptor dimerization in living cells

using bioluminescence resonance energy transfer (BRET)," Proc. Natl, Acad. Sci. USA 97, 7: 3684 - 3689.

[00124] The present inventors have determined that these modified GPCRs are useful in agonist-independent assays for screening compounds that may alter GPCR internalization. Examples of assays in which the present invention may be used include, but are not limited to, assays described herein.

Methods of enhancing GPCR desensitization

[00125] Provided in the present invention are methods of enhancing GPCR desensitization. One embodiment is related to the expression of GRKs, which may be modified. The GRKs may be over-expressed or their expression may be inducible. These methods may be used to analyze the desensitization of a GPCR, including a modified GPCR, an orphan GPCR, a taste receptor, a mutant GPCR, the β2AR Y326A GPCR mutant, or another GPCR. Certain GPCRs useful in the present invention are listed in Figure 1.

[00126] In a preferred embodiment, a cell is provided that contains an expression system and a nucleic acid encoding a GRK. The GRK may be modified such that the expression of the GRK results in constitutive desensitization of the GPCR. The GRK may be over-expressed and its expression may be inducible.

[00127] Preferably, host cells are provided which include a GRK, which may be modified, and arrestin. A GPCR is then added to these cells. The agonist-independent desensitization of the GPCR is detected. Figures 4, 5, 6, and 7 are examples of this method. Detection methods are described below.

[00128] The present invention provides methods of determining if the GPCR of interest is expressed at the plasma membrane. GPCRs expressed at the plasma membrane are useful in the previously mentioned methods of compound identification.

[00129] A preferred method of determining if a GPCR of interest is expressed at the plasma membrane includes: (a) providing a cell including a GPCR, an arrestin, and a GRK, wherein the arrestin is detectably labeled; (b) determining the cellular distribution of the arrestin; and (c) correlating the cellular distribution of the arrestin to the ability of the GPCR to be expressed at the plasma membrane.

[00130] Preferred embodiments of this aspect of the invention are described in Examples 2, 3, 4, 5, 6, and 7 and illustrated in Figures 4, 5, 6, and 7.

[00131] Another method of determining if a GPCR of interest is expressed at the plasma membrane includes: (a) providing a cell comprising a GPCR and a GRK, wherein the GRK is detectably labeled; (b) determining the cellular distribution of the GRK; and (c) correlating the cellular distribution of the GRK to the ability of the GPCR to be expressed at the plasma membrane.

[00132] The present invention provides methods of analyzing the ability of a GPCR to bind arrestin. GPCRs which bind arrestin are useful in the previously mentioned methods of compound identification.

[00133] A preferred method of analyzing the ability of a GPCR to bind arrestin includes: (a) providing a cell including a GPCR, an arrestin, and a GRK, wherein the arrestin is detectably labeled; (b) determining the cellular distribution of the arrestin; and (c) correlating the cellular distribution of the arrestin to the ability of the GPCR to bind arrestin.

[00134] Preferred embodiments of this aspect of the invention are described in Examples 2, 3, 4, 5, 6, and 7, and illustrated in Figures 4, 5, 6, and 7.

[00135] Using this method, certain GPCRs will bind arrestin and desensitize. However, certain GPCRs will not desensitize without modification of the GPCR, as described in U.S.S.N. 09/993,844. The present inventors modified several GPCRs, including known and orphan

GPCRs, listed in Figure 3. Upon modification, these modified GPCRs constitutively desensitized in the above system.

Modified GPCRs

[00136] The present invention is related to modified GPCRs. Modified GPCRs of the present invention may comprise one or more modifications in their carboxyl-terminal tail. These modifications may comprise inserting one or more sites of phosphorylation, preferably clusters of phosphorylation sites, within certain regions of the carboxyl-terminal tail. As such, the carboxyl-terminal tail may be modified in whole or in part. The carboxyl-terminal tail of many GPCRs begins shortly after a conserved NPXXY motif that marks the end of the seventh transmembrane domain (i.e. what follows the NPXXY motif is the carboxyl-terminal tail of the GPCR). The carboxyl-terminal tail of many GPCRs comprises a putative site of palmitoylation approximately 10 to 25 amino acid residues, preferably 15 to 20 amino acid residues, downstream of the NPXXY motif. This site is typically one or more cysteine residues. The carboxyl-terminal tail of a GPCR may be relatively long, relatively short, or virtually non-existent. The present inventors have determined that the carboxyl-terminal tail of a GPCR determines the affinity of arrestin binding.

[00137] The present inventors have discovered that specific amino acid motifs in the carboxyl-terminal tail promote formation of a stable GPCR/arrestin complex and thus ultimately may promote recruitment of arrestin to endosomes. These amino acid motifs comprise one or more amino acids, preferably clusters of amino acid residues, that may be efficiently phosphorylated and thus readily function as phosphorylation sites. The clusters of amino acids may occupy two out of two, two out of three, three out of three, three out of four positions, four out of four, four out of five positions, five out of five, and the like consecutive amino acid positions. Accordingly, the clusters of amino acids that promote formation of a stable

GPCR/arrestin complex are "clusters of phosphorylation sites." These clusters of phosphorylation sites are preferably clusters of serine and threonine residues.

[00138] GPCRs that form stable complexes with arrestin comprise one or more sites of phosphorylation, preferably clusters of phosphorylation sites. In addition to the presence of the one or more sites of phosphorylation, preferably clusters of phosphorylation sites, it has been discovered that the sites must be properly positioned within the carboxyl-terminal tail to promote formation of a stable GPCR/arrestin complex. To promote formation of a stable GPCR/arrestin complex, the one or more sites of phosphorylation, preferably one or more clusters of phosphorylation, may be approximately 15 to 35 (preferably 15 to 25) amino acid residues downstream of a putative site of palmitoylation of the GPCR. In addition, the one or more sites of phosphorylation, preferably one or more clusters of phosphorylation, may be approximately 20 to 55 (preferably 30 to 45) amino acid residues downstream of the NPXXY motif of the GPCR. GPCRs containing one or more sites of phosphorylation, preferably clusters of phosphorylation sites, properly positioned are typically Class B receptors.

[00139] By way of example, it has been discovered that the V2R receptor comprises a cluster of phosphorylation sites (SSS) that promotes formation of a stable GPCR/arrestin complex at 19 amino acid residues downstream of the putative site of palmitoylation and 36 amino acid residues downstream of the NPXXY motif. The NTR-2 receptor comprises a cluster of phosphorylation sites (STS) that promotes formation of a stable GPCR/arrestin complex at 26 amino acid residues downstream of the putative site of palmitoylation and 45 amino acid residues downstream of the NPXXY motif. The oxytocin receptor (OTR) receptor comprises two clusters of phosphorylation sites (SSLST and STLS) that promote formation of a stable GPCR/arrestin complex, one at 20 amino acid residues downstream of the putative site of palmitoylation and the other at 29 amino

acid residues downstream of the putative site of palmitoylation, and one at 38 amino acid residues downstream of the NPXXY motif and the other at 47 amino acid residues downstream of the NPXXY motif, respectively. The substance P receptor (SPR, also known as the neurokinin-1 receptor) comprises a cluster of phosphorylation sites (TTIST) that promotes formation of a stable GPCR/arrestin complex at 32 amino acid residues downstream of the putative site of palmitoylation and 50 amino acid residues downstream of the NPXXY motif.

[00140] The present inventors have determined that GPCRs that lack one or more sites of phosphorylation, preferably clusters of phosphorylation, properly positioned within the carboxyl terminal tail form GPCR/arrestin complexes that are less stable and dissociate at or near the plasma membrane. These GPCRs are typically Class A receptors, olfactory receptors, taste receptors, and the like. However, the present inventors have discovered that stable GPCR/arrestin complexes may be achieved with GPCRs naturally lacking one or more sites of phosphorylation and having a lower affinity for arrestin by modifying the carboxyl-terminal tails of these receptors. Preferably, the carboxyl-terminal tails are modified to include one or more sites of phosphorylation, preferably one or more clusters of phosphorylation sites, properly positioned within the carboxyl terminal tail.

[00141] The present invention includes the polypeptide sequences of these modified GPCRs. The modified GPCRs of the present invention include GPCRs that have been modified to have one or more sites of phosphorylation, preferably one or more clusters of phosphorylation, properly positioned in their carboxyl terminal tails. The polypeptide sequences of the modified GPCRs of the present invention also include sequences having one or more additions, deletions, substitutions, or mutations. These mutations are preferably substitution mutations made in a conservative manner (i.e., by changing the codon from an amino acid

belonging to a grouping of amino acids having a particular size or characteristic to an amino acid belonging to the same grouping). Such a conservative change generally leads to less change in the structure and function of the resulting protein. The present invention should be considered to include sequences containing conservative changes which do not significantly alter the activity or binding characteristics of the resulting protein.

[00142] The modified GPCRs of the present invention include GPCRs containing a NPXXY motif, a putative site of palmitoylation approximately 10 to 25 amino acid residues (preferably 15 to 20 amino acids) downstream of the NPXXY motif, and a modified carboxyl-terminal tail. The modified carboxyl-terminal tail has one or more sites of phosphorylation, preferably one or more clusters of phosphorylation sites, such that the phosphorylation sites are approximately 15 to 35, preferably 15 to 25, amino acid residues downstream of the putative site of palmitoylation of the modified GPCR. The modified carboxyl-terminal tail may have one or more sites of phosphorylation, preferably one or more clusters of phosphorylation sites, such that the phosphorylation sites are approximately 20 to 55, preferably 30 to 45, amino acid residues downstream of the NPXXY of the modified GPCR.

[00143] The present invention further includes isolated nucleic acid molecules that encode modified GPCRs. It should be appreciated that also within the scope of the present invention are DNA sequences encoding modified GPCRs which code for a modified GPCR having the same amino acid sequence as the modified GPCRs, but which are degenerate. By "degenerate to" it is meant that a different three-letter codon is used to specify a particular amino acid.

[00144] As one of skill in the art would readily understand, the carboxyl-tail of many GPCRs may be identified by the conserved NPXXY motif that marks the end of the seventh transmembrane domain.

[00145] To create a modified GPCR containing a modified carboxyl-terminus region according to the present invention, a GPCR lacking phosphorylation sites or clusters of phosphorylation sites or with a lower or unknown affinity for arrestin may have one or more additions, substitutions, deletions, or mutations of amino acid residues in its carboxyl-terminal tail. These additions, substitutions, deletions, or mutations are performed such that the carboxyl-terminal tail is modified to comprise one or more sites of phosphorylation, preferably clusters of phosphorylation sites. By way of example, discrete point mutations of the amino acid residues may be made to provide a modified GPCR. By way of example three consecutive amino acids may be mutated to serine residues to provide a modified GPCR. These mutations are made such that the one or more sites of phosphorylation, preferably clusters of phosphorylation sites, are properly positioned within the carboxyl terminal tail.

[00146] In addition, to create a modified GPCR containing a modified carboxyl-terminal tail region, mutations may be made in a nucleic acid sequence of a GPCR lacking sites of phosphorylation or clusters of phosphorylation sites or with a lower or unknown affinity for arrestin such that a particular codon is changed to a codon which codes for a different amino acid, preferably a serine or threonine. Such a mutation is generally made by making the fewest nucleotide changes possible. A substitution mutation of this sort can be made to change an amino acid in the resulting protein to create one or more sites of phosphorylation, preferably clusters of phosphorylation sites. Also by way of example, discrete point mutations of the nucleic acid sequence may be made. The phosphorylation sites are positioned such that they are located approximately 15 to 35 amino acid residues downstream of the putative site of palmitoylation of the modified GPCR.

[00147] Furthermore, to provide modified GPCRs of the present invention, a GPCR lacking properly positioned phosphorylation sites or with a lower or

unknown affinity for arrestin may also have its carboxyl-terminal tail, in whole or in part, exchanged with that of a GPCR having properly positioned clusters of phosphorylation sites. The site of exchange may be after or including the conserved NPXXY motif. As an alternative, a putative site of palmitoylation of a GPCR may be identified at approximately 10 to 25 (preferably 15 to 20) amino acid residues downstream of the conserved NPXXY motif, and the site of exchange may be after or including the palmitoylated cysteine(s). Preferably, the carboxyl-terminal tail of a GPCR lacking properly positioned clusters of phosphorylation sites or with a lower or unknown affinity for arrestin is exchanged at an amino acid residue in close proximity to a putative site a palmitoylation. More preferably, the carboxyl-terminal tail of a GPCR lacking properly positioned clusters of phosphorylation sites or with a lower or unknown affinity for arrestin is exchanged at a putative site of palmitoylation approximately 10 to 25 (preferably 15 to 20) amino acid residues downstream of the NPXXY motif, such that the palmitoylated cysteine residue is maintained. Exchanging in the preferred manner allows the clusters of phosphorylation sites to be reliably positioned properly within the carboxyl-terminal tail of the modified GPCR. The tails may be exchanged and the modified GPCRs may be constructed accordingly by manipulation of the nucleic acid sequence or the corresponding amino acid sequence.

[00148] In a further alternative, the carboxyl-tail of a GPCR, for example a GPCR not containing the NPXXY motif, may be predicted from a hydrophobicity plot and the site of exchange may be selected accordingly. Based on a hydrophobicity plot, one of skill in the art may predict a site where it is expected that the GPCR may anchor in the membrane and then predict where to introduce a putative site of palmitoylation accordingly. Using this technique GPCRs having neither a NPXXY motif nor a putative site of palmitoylation may be modified to create a point of reference (e.g. a putative site of palmitoylation). The introduced putative site of palmitoylation

may then be used to position a tail exchange.

[00149] The carboxyl-terminal tail used for the exchange may be from a second GPCR having one or more properly positioned clusters of phosphorylation sites and having a putative site of palmitoylation approximately 10 to 25 (preferably 15 to 20) amino acid residues downstream of a NPXXY motif. The tail as identified may be exchanged, after or including the conserved NPXXY motif. As an alternative, a putative site of palmitoylation of a GPCR may be identified at approximately 10 to 25 (preferably 15 to 20) amino acid residues downstream of the conserved NPXXY motif, and the tail may be exchanged after or including the palmitoylated cysteine(s). Preferably, the carboxyl-terminal tail of a GPCR having clusters of phosphorylation sites is exchanged at an amino acid residue in close proximity to a putative site of palmitoylation. More preferably, the carboxyl-terminal tail of a GPCR having clusters of phosphorylation sites is exchanged at a putative site of palmitoylation approximately 10 to 25 (preferably 15 to 20) amino acid residues downstream of the NPXXY motif, such that the portion of the carboxyl-terminal tail containing the clusters of phosphorylation sites begins at the amino acid residue immediately downstream of the palmitoylated cysteine residue. Exchanging in the preferred manner allows the clusters of phosphorylation sites to be reliably positioned properly within the carboxyl-terminal tail of the modified GPCR. The carboxyl-terminal tail having clusters of phosphorylation sites used for the exchange may have a detectable molecule conjugated to the carboxyl-terminus. The tails may be exchanged and the modified GPCRs may be constructed accordingly by manipulation of the nucleic acid sequence or the corresponding amino acid sequence.

[00150] In addition, the carboxyl-terminal tail portion used for the exchange may originate from a polypeptide synthesized to have an amino acid sequence corresponding to an amino acid sequence from a GPCR

having one or more sites of phosphorylation, preferably one or more clusters of phosphorylation sites. The synthesized polypeptide may have a putative site of palmitoylation approximately 10 to 25 (preferably 15 to 20) amino acid residues downstream of a NPXXY motif. The synthesized polypeptide may have one or more additions, substitutions, mutations, or deletions of amino acid residues that does not affect or alter the overall structure and function of the polypeptide.

[00151] Furthermore, the carboxyl-terminal tail portion used for the exchange may originate from a naturally occurring polypeptide recognized to have an amino acid sequence corresponding to an amino acid sequence from a GPCR having one or more clusters of phosphorylation sites. The polypeptide may have a putative site of palmitoylation approximately 10 to 25 (preferably 15 to 20) amino acid residues downstream of a NPXXY motif. The polypeptide may have one or more additions, substitutions, mutations, or deletions of amino acid residues that does not affect or alter the overall structure and function of the polypeptide.

[00152] A modified GPCR containing a modified carboxyl-terminus region may be created by fusing a first carboxyl-terminal tail portion of a GPCR lacking properly positioned clusters of phosphorylation sites or with a lower or unknown affinity for arrestin with a second carboxyl-terminal tail portion of a GPCR or polypeptide having one or more clusters of phosphorylation sites. The second GPCR or polypeptide used for the exchange may have a putative site of palmitoylation approximately 10 to 25 (preferably 15 to 20) amino acid residues downstream of a NPXXY motif. Accordingly, the modified carboxyl-terminus region of the modified GPCR comprises a portion of a carboxyl-terminal tail from a GPCR lacking properly positioned clusters of phosphorylation sites or with a lower or unknown affinity for arrestin fused to a portion of a carboxyl-terminal tail of a GPCR or polypeptide having clusters of phosphorylation sites. The tail of a GPCR lacking properly positioned clusters of phosphorylation sites may be

exchanged after or including the conserved NPXXY motif, and fused to a carboxyl-terminal tail containing clusters of phosphorylation sites, after or including the conserved NPXXY motif. As an alternative, the tail of a GPCR lacking properly positioned clusters of phosphorylation sites may be exchanged after or including the palmitoylated cysteine(s), and fused to a tail containing clusters of phosphorylation sites, after or including the palmitoylated cysteine(s). The tails may be exchanged and the modified GPCRs may be constructed accordingly by manipulation of the nucleic acid sequence or the corresponding amino acid sequence.

[00153] In a further alternative, the carboxyl-tail of a GPCR, for example a GPCR not containing the NPXXY motif, may be predicted from a hydrophobicity plot and exchanged accordingly. The site of exchange may be selected according to the hydrophobicity plot. Based on a hydrophobicity plot, one of skill in the art may predict a site where it is expected that the GPCR may anchor in the membrane and then predict where to introduce a putative site of palmitoylation accordingly. Using this techique GPCRs having neither a NPXXY motif nor a putative site of palmitoylation may be modified to create a point of reference (e.g. a putative site of palmitoylation). The introduced putative site of palmitoylation may be then used to position a tail exchange. After introduction of a putative site of palmitoylation, the resulting tail may be fused with a second carboxyl-terminal tail portion of a GPCR or polypeptide having one or more clusters of phosphorylation sites and having a putative site of palmitoylation approximately 10 to 25 (preferably 15 to 20) amino acid residues downstream of a NPXXY motif.

[00154] Preferably, the modified carboxyl-terminus region of the modified GPCR is fused at amino acid residues in close proximity to a putative site of palmitoylation. More preferably, the modified carboxyl-terminus region of the modified GPCR is fused such that the portion from the first GPCR with a lower affinity for arrestin comprises amino acid residues from the NPXXY motif through a putative site of palmitoylation approximately 10 to 25

(preferably 15 to 20) amino acid residues downstream of the NPXXY motif and the portion from the second GPCR having clusters of phosphorylation sites and a putative site of palmitoylation approximately 10 to 25 (preferably 15 to 20) amino acid residues downstream of a NPXXY motif comprises amino acid residues beginning with an amino acid residue immediately downstream of the putative site of palmitoylation of the second GPCR extending to the end of the carboxyl-terminus. This fusion is preferred because the clusters of phosphorylation sites are reliably positioned properly within the carboxyl-terminal tail and the modified GPCR maintains its structure and ability to function.

[00155] By way of example, a Class A receptor or an orphan receptor may have a portion of its carboxyl-terminal tail exchanged with a portion of a carboxyl-terminal tail from a known Class B receptor. Further, receptors having virtually non-existent carboxyl-terminal tails, for example, olfactory receptors and taste receptors, may have a portion of their carboxyl-terminal tails exchanged with a portion of a carboxyl-terminal tail from a known Class B receptor. The Class B receptor tail used for these exchanges may have a detectable molecule fused to the carboxyl-terminus.

[00156] Modified GPCRs may be generated by molecular biological techniques standard in the genetic engineering art, including but not limited to, polymerase chain reaction (PCR), restriction enzymes, expression vectors, plasmids, and the like. By way of example, vectors, such as a pEArrB (enhanced arrestin binding), may be designed to enhance the affinity of a GPCR lacking clusters of phosphorylation sites for arrestin. To form a vector, such as a pEArrB vector, PCR amplified DNA fragments of a GPCR carboxyl-terminus, which forms stable complexes with arrestin, may be digested by appropriate restriction enzymes and cloned into a plasmid. A schematic of one such plasmid is illustrated in Figure 4A. The DNA of a GPCR, which is to be modified, may also be PCR amplified, digested by restriction enzymes at an appropriate location, and subcloned into the

vector, such as pEArrB, as illustrated in Figure 4B. When expressed, the modified GPCR will contain a polypeptide fused to the carboxyl-terminus. The polypeptide will comprise clusters of phosphorylation sites. Preferably, the polypeptide originates from the GPCR carboxyl-terminus of a receptor that forms stable complexes with arrestin.

[00157] Such modified GPCRs may also occur naturally as the result of aberrant gene splicing or single nucleotide polymorphisms. Such naturally occurring modified GPCRs would be predicted to have modified endocytic targeting. These naturally occurring modified GPCRs may be implicated in a number of GPCR-related disease states.

[00158] As shown in Figure 3, the present inventors modified several GPCRs. The β2-adrenergic receptor, dopamine D1A receptor, mu opiod receptor, orphan GPR3, orphan GPR6, orphan GPR12, orphan GPR7, orphan GPR8, orphan GPR55, orphan SREB2, and orphan SREB3 were modified as described herein. These modified GPCRs contain a properly positioned V2R cluster of phosphorylation sites (SSS) within the modified GPCR's tail.

[00159] As may be shown by standard receptor binding assays, the modified receptors are essentially indistinguishable from their wild-type counterparts except for an increased affinity for arrestin and thus an increased stability of their complex with arrestin and in their ability to traffic with arrestin and in their ability to recycle and resensitize. For example, the modified receptors are appropriately expressed at the membrane and possess similar affinity for agonists or ligands. However, the modified GPCRs have an increased affinity for arrestin and thus form a more stable complex with arrestin than their wild-type counterparts and may remain bound to arrestin when trafficking to endosomes.

[00160] These modified GPCRs are useful in assays to screen for an agonist of the GPCR, as well as in agonist-independent assays to identify compounds that alter GPCR desensitization.

<u>Methods of Assaying GPCR Activity using the Modified GPCRs</u>

[00161] The modified GPCRs of the present invention are useful in methods of assaying GPCR activity. The modified GPCRs of the present invention may be used in assays to study GPCRs that have weaker than desired interactions or associations with arrestins and GPCRs that have unknown interactions or associations with arrestins. Methods of the present invention that use the modified GPCRs provide a sensitive assay and may provide for enhanced detection, for example, of arrestin/GPCRs in endosomes. The assays using the modified GPCRs of the present invention may be useful for screening compounds and sample solutions for ligands, agonists, antagonists, inverse agonists, desensitization active compounds, and the like. Once identified, these compounds may be useful as drugs capable of modulating GPCR activity and useful in the treatment of one or more of the disease states in which GPCRs have been implicated.

[00162] In a preferred assay according to the present invention, cells are provided that express modified GPCRs of the present invention and these cells may further contain a conjugate of an arrestin and a detectable molecule.

[00163] Arrestin coupled to a detectable molecule may be detected and monitored as it functions in the GPCR pathway. The location of the arrestin may be detected, for example, evenly distributed in the cell cytoplasm, concentrated at a cell membrane, concentrated in clathrin-coated pits, localized on endosomes, and the like. In response to agonist stimulation, the proximity of arrestin to a GPCR may be monitored, as well as the proximity to any other cell structure. For example, in response to agonist stimulation arrestin may be detected in proximity to GPCRs at a cell membrane, concentrated with GPCRs in clathrin-coated pits, colocalized with a GPCR on endosomes, and the like.

[00164] The modified GPCRs of the present invention have an increased

affinity for arrestin and provide a stable complex of the GPCR with arrestin, and thereby promote colocalization of the GPCR with arrestin into endosomes. In the methods of assaying of the present invention, arrestin may be detected, for example, in the cytoplasm, concentrated in proximity to GPCRs at a cell membrane, concentrated in proximity to GPCRs in clathrin-coated pits, colocalized with a GPCR on endosomes, and the like. Preferably the arrestin may be detected colocalized with a GPCR on endosomes.

[00165] The association of arrestin with a GPCR at a cell membrane may be rapidly detected after agonist addition, for example, approximately 1 second to 2 minutes. The colocalization of arrestin with GPCR on endosomes may be detected within several minutes of agonist addition, for example, approximately 3 to 15 minutes, and may persist for extended periods of time, for example, after 1 hour. The association of arrestin with GPCR on endosomes may give a strong, readily recognizable signal. Under magnification of 40X objective lens, the signal may be doughnut-like in appearance. The signal resulting from the compartmentalization of arrestin and GPCR colocalized in endosomes vesicles is typically easy to detect and may persist for extended periods of time.

[00166] A preferred method of assessing GPCR pathway activity of the present invention comprises (a) providing a cell that expresses at least one modified GPCR of the present invention and that further comprises a conjugate of an arrestin and a detectable molecule; (b) inducing translocation of the arrestin; and (c) detecting interaction of the arrestin with the modified GPCR along the translocation pathway.

[00167] Interaction of the arrestin with the modified GPCR may be detected, for example, in endosomes, in clathrin-coated pits, concentrated in proximity to a cell membrane, and the like. Preferably, interaction of the arrestin with the modified GPCR is detected in endosomes. Interaction of arrestin with a GPCR in endosomes may be detected within several minutes

of agonist addition, for example, approximately 3 to 15 minutes, and may persist for extended periods of time, for example, after 1 hour. The association of arrestin with a GPCR in endosomes may give a strong, readily recognizable signal that persists for extended periods of time.

[00168] In a method of screening compounds for GPCR activity of the present invention a cell that expresses at least one modified GPCR is provided. The cell further contains arrestin conjugated to a detectable molecule. The cell is exposed to the compounds to be tested. The location of the arrestin within the cell is detected. The location of the arrestin within the cell in the presence of the compound is compared to the location of the arrestin within the cell in the absence of the compound, and a difference is correlated between (1) the location of the arrestin within the cell in the presence of the compound and (2) the location of the arrestin within the cell in the absence of the compound.

[00169] By way of example, compounds and sample solutions may be screened for GPCR agonist activity using the modified GPCRs of the present invention. In this method, cells that express at least one modified GPCR of the present invention and that further comprise a conjugate of an arrestin and a detectable molecule are provided. The cells are exposed to compounds or sample solutions to be tested. It is detected whether interaction of the arrestin with the modified GPCR is increased after exposure to the test compound or solution, an increase in interaction being an indication that the compound or solution has GPCR agonist activity. Interaction of the arrestin with the GPCR may be detected in endosomes, in clathrin-coated pits, in proximity to a cell membrane, and the like. The modified GPCR may also be conjugated to a detectable molecule, preferably at the carboxyl-terminus. As explained above modifications to GPCRs as in the present invention should not affect the GPCRs' natural affinity for agonists or ligands.

[00170] Also by way of example, compounds and sample solutions may

be screened for GPCR antagonist or inverse agonist activity using the modified GPCRs of the present invention. Cells that express at least one modified GPCR of the present invention and that further comprise a conjugate of an arrestin and a detectable molecule are provided. The cells are exposed to compounds or sample solutions to be tested and to a known agonist for the GPCR. It is detected whether interaction of the arrestin with the modified GPCR is decreased after exposure to the test compound or solution, a decrease in interaction being an indication that the compound or solution has GPCR antagonist or inverse agonist activity. Interaction of the arrestin with the GPCR may be detected in endosomes, in clathrin-coated pits, in proximity to a cell membrane, and the like. The modified GPCR may also be conjugated to a detectable molecule, preferably at the carboxyl-terminus. As explained above modifications to GPCRs as in the present invention should not affect the GPCRs' natural affinity for antagonists or inverse agonists.

[00171] Further by way of example, compounds and sample solutions may be screened for GPCR desensitization activity using the modified GPCRs of the present invention. First cells that express at least one first modified GPCR of the present invention and that further comprise a conjugate of an arrestin and a detectable molecule are provided. The first cells are exposed to compounds or sample solutions to be tested and to a known agonist for the first GPCR. It is detected whether interaction of the arrestin with the first modified GPCR is decreased or not increased after exposure to the test compound or solution, a decrease or lack of increase in interaction being an indication that the compound or solution has GPCR desensitization activity. Interaction of the arrestin with the GPCR may be detected in endosomes, in clathrin-coated pits, in proximity to a cell membrane, and the like. Then second cells that express at least one second modified GPCR of the present invention and that further comprise a conjugate of an arrestin and a detectable molecule are provided. The second modified GPCR is not

related to the first modified GPCR. The second cells are exposed to the compounds or sample solutions to be tested and to a known agonist for the second GPCR. It is detected whether interaction of the arrestin with the second modified GPCR is decreased or not increased after exposure to the test compound or solution, a decrease or lack of increase in interaction being an indication that the compound or solution has GPCR desensitization activity independent of the GPCR expressed. Interaction of the arrestin with the GPCR may be detected in endosomes, in clathrin-coated pits, in proximity to a cell membrane, and the like.

[00172] The methods of assessing GPCR pathway activity of the present invention also include cell-free assays. In cell-free assays of the present invention, a substrate having deposited thereon a modified GPCR of the present invention is provided. A fluid containing a conjugate of an arrestin and a detectable molecule is also provided. Translocation of the arrestin is induced and interaction of the arrestin with the GPCR is detected. The GPCR and arrestin may be obtained from whole cells and used in the cell-free assay after purification. The modified GPCR has arrestin binding sites and agonist binding sites and may be supported in a multilayer or bilayer lipid vesicle. The vesicle supporting the modified GPCR may be deposited on the substrate, and the modified GPCR may be supported in the lipid vesicle and deposited on the substrate such that the arrestin binding sites are exposed to arrestin and the receptor binding sites are accessible to agonists. The substrate may be any artificial substrate on which the GPCR may be deposited, including but not limited to, glass, plastic, diamond, ceramic, semiconductor, silica, fiber optic, diamond, biocompatible monomer, biocompatible polymer, polymer beads (including organic and inorganic polymers), and the like.

[00173] The present invention relates to the compounds identified as ligands, agonists, antagonists, inverse agonists, or DACs by the methods of assaying of the present invention. These compounds may be used to treat

any one of the disease states in which GPCRs have been implicated. The compounds identified may be administered to a human or a non-human in therapeutically effective doses to treat or ameliorate a condition, disorder, or disease in which GPCRs have been implicated. A therapeutically effective dose refers to that amount of the compound sufficient to result in amelioration of symptoms of such a condition, disorder or disease.

Methods to identify compounds that modulate GPCR desensitization

[00174] The present invention relates to methods of screening for compounds that modulate GPCR desensitization. The methods utilize modified GRKs which constitutively phosphorylate GPCRs, resulting in constitutive desensitization. These may be used to identify compounds that alter the desensitization of GPCRs, even if the GPCR agonist is unknown. Once identified, these compounds may be useful as drugs capable of modulating GPCR activity and useful in the treatment of one or more of the disease states in which GPCRs have been implicated.

[00175] In a preferred method according to the present invention, cells are provided that contain an expression system and a nucleic acid encoding a modified GRK, resulting in constitutive desensitization of GPCRs expressed in the cell. These cells may further contain an arrestin conjugated to a GFP.

[00176] A preferred method of identifying a compound which inhibits GPCR internalization includes: (a) providing a cell including a GPCR, an arrestin, and a modified GRK; (b) exposing the cell to the compound(s); (c) determining the cellular distribution of the GPCR or arrestin; and (d) correlating a difference between (1) the location of the labeled molecule in the cell in the presence of the compound(s) and (2) the location of the labeled molecule in the cell in the absence of the compound(s) to modulation of GPCR internalization. Non-limiting embodiments of this method are described in Figures 4, 5, 6, and 7 and Examples 2, 3, 4, 5, 6, and 7.

[00177] The GRK of step (a), as described above, may be GRK 1, 2, 3, 4, 5, 6, or any other GRK, including splice variants, biologically active fragments, or modified GRKs. The GRK may be overexpressed and/or its expression may be inducible. The GRK may include a CAAX motif.

[00178] In the above method, agonist may or may not be provided.

[00179] Methods of detecting the labeled molecules and determining the cellular distribution of the GPCR or arrestin are described below.

[00180] GPCRs useful in the present invention include, but are not limited to GPCRs which have known agonists, GPCRs which do not have known agonists, GPCRs listed in Figure 1, GPCRs illustrated in Figures 3, 4, 5, 6, and 7, Class A GPCRs, Class B GPCRs, taste receptors, odorant receptors, orphan receptors, modified GPCRs, GPCRs as described in U.S. Patent Application Nos. 10/054,616, 09/993,844, 10/095,620, 10/101,235, 09/631,468, 10/141,725, 10/161,916, 09/469,554, 09/772,644, 60/393,789, and 60/379,986, which are herein incorporated by reference, or biologically active fragments of the above GPCRs.

<u>Vectors and nucleic acids, host cells for protein expression</u>

[00181] The present invention relates to modified GRKs, including GRKs which are over-expressed, or their expression is inducible.

[00182] Nucleic acids encoding modified GRKs are provided. The present invention relates to the expression, over-expression, and the inducible expression of these proteins. The expression may be carried out by a suitable expression system contained in a vector, as described below.

[00183] One aspect of the present invention relates to the combination of (1) nucleic acids encoding a modified GRK with (2) a system for expression of modified GRKs resulting in constitutive desensitization of GPCRs. This system for expression of modified GRKs may include a promoter or origin of replication.

[00184] Another aspect of the present invention relates to modified

GPCRs, nucleic acids encoding modified GPCRs, and host cell for modified GPCR expression.

[00185] Nucleic acids encoding modified GPCRs are provided. The present invention relates to the expression, over-expression, and the inducible expression of these proteins. The expression may be carried out by a suitable expression system contained in a vector, as described below.

[00186] A feature of this invention is the expression of the DNA sequences disclosed herein. As is well known in the art, DNA sequences may be expressed by operatively linking them to an expression control sequence in an appropriate expression vector and employing that expression vector to transform an appropriate unicellular host. The transforming DNA may or may not be integrated (covalently linked) into chromosomal DNA making up the genome of the cell.

[00187] Such operative linking of a DNA sequence of this invention to an expression control sequence, of course, includes, if not already part of the DNA sequence, the provision of an initiation codon, ATG, in the correct reading frame upstream of the DNA sequence.

[00188] A wide variety of host/expression vector combinations may be employed in expressing the DNA sequences of this invention. Useful expression vectors, for example, may consist of segments of chromosomal, non-chromosomal and synthetic DNA sequences. Suitable vectors include derivatives of SV40 and known bacterial plasmids, e.g., *E. coli* plasmids col EI, pCR1, pBR322, pMB9 and their derivatives, plasmids such as RP4; phage DNAS, e.g., the numerous derivatives of phage λ, e.g., NM989, and other phage DNA, e.g., M13 and filamentous single stranded phage DNA; yeast plasmids such as the 2μ plasmid or derivatives thereof; vectors useful in eukaryotic cells, such as vectors useful in insect or mammalian cells; vectors derived from combinations of plasmids and phage DNAs, such as plasmids that have been modified to employ phage DNA or other expression control sequences; and the like.

[00189] Any of a wide variety of expression control sequences -- sequences that control the expression of a DNA sequence operatively linked to it -- may be used in these vectors to express the DNA sequences of this invention. Such useful expression control sequences include, for example, the early or late promoters of SV40, CMV, vaccinia, polyoma or adenovirus, the lac system, the trp system, the TAC system, the TRC system, the LTR system, the major operator and promoter regions of phage λ, the control regions of fd coat protein, the promoter for 3-phosphoglycerate kinase or other glycolytic enzymes, the promoters of acid phosphatase (e.g., Pho5), the promoters of the yeast α-mating factors, and other sequences known to control the expression of genes of prokaryotic or eukaryotic cells or their viruses, and various combinations thereof.

[00190] A wide variety of unicellular host cells are also useful in expressing the DNA sequences of this invention. These hosts may include well known eukaryotic and prokaryotic hosts, such as strains of *E. coli*, *Pseudomonas*, *Bacillus*, *Streptomyces*, fungi such as yeasts, plant cells, nematode cells, and animal cells, such as HEK-293, U2OS, CHO, RI.I, B-W and L-M cells, African Green Monkey kidney cells (e.g., COS 1, COS 7, BSC1, BSC40, and BMT10), insect cells (e.g., Sf9), and human cells and plant cells in tissue culture. In one aspect of the present invention, the host cells include a GRK-C20 and an arrestin. In a further aspect of the present invention, the host cells include a GRK-C20, an arrestin, and a GPCR.

[00191] It will be understood that not all vectors, expression control sequences and hosts will function equally well to express the DNA sequences of this invention. Neither will all hosts function equally well with the same expression system. However, one skilled in the art will be able to select the proper vectors, expression control sequences, and hosts without undue experimentation to accomplish the desired expression without departing from the scope of this invention. For example, in selecting a vector, the host must be considered because the vector must function in it.

The vector's copy number, the ability to control that copy number, and the expression of any other proteins encoded by the vector, such as antibiotic markers, will also be considered.

[00192] In selecting an expression control sequence, a variety of factors will normally be considered. These include, for example, the relative strength of the system, its controllability, and its compatibility with the particular DNA sequence or gene to be expressed, particularly as regards potential secondary structures. Suitable unicellular hosts will be selected by consideration of, e.g., their compatibility with the chosen vector, their secretion characteristics, their ability to fold proteins correctly, and their fermentation requirements, as well as the toxicity to the host of the product encoded by the DNA sequences to be expressed, and the ease of purification of the expression products.

[00193] Considering these and other factors a person skilled in the art will be able to construct a variety of vector/expression control sequence/host combinations that will express the DNA sequences of this invention on fermentation or in large scale animal culture.

[00194] It is further intended that modified GRK analogs may be prepared from nucleotide sequences of the protein complex/subunit derived within the scope of the present invention. Analogs, such as fragments, may be produced, for example, by pepsin digestion of GRK material. Other analogs, such as muteins, can be produced by standard site-directed mutagenesis of GRK coding sequences. Analogs exhibiting "GRK activity" such as small molecules, whether functioning as promoters or inhibitors, may be identified by known *in vivo* and/or *in vitro* assays.

[00195] As mentioned above, a DNA sequence encoding a modified GRK6 can be prepared synthetically rather than cloned. The DNA sequence can be designed with the appropriate codons for the GRK amino acid sequence. In general, one will select preferred codons for the intended host if the sequence will be used for expression. The complete sequence is

assembled from overlapping oligonucleotides prepared by standard methods and assembled into a complete coding sequence. See, e.g., Edge, Nature, 292:756 (1981); Nambair et al., Science, 223:1299 (1984); Jay et al., J. Biol. Chem., 259:6311 (1984).

[00196] Synthetic DNA sequences allow convenient construction of genes which will express GRK analogs or "muteins". Alternatively, DNA encoding muteins can be made by site-directed mutagenesis of native or modified GRK genes or cDNAs, and muteins can be made directly using conventional polypeptide synthesis.

[00197] A general method for site-specific incorporation of unnatural amino acids into proteins is described in Christopher J. Noren, Spencer J. Anthony-Cahill, Michael C. Griffith, Peter G. Schultz, Science, 244:182-188 (April 1989). This method may be used to create analogs with unnatural amino acids.

[00198] Additional motifs, such as epitope tags or sequences to aid in purification, may be incorporated into the nucleic acids encoding the modified GRKs or modified GPCRs. Preferably, the nucleic acids encoding the motifs may be at the 5' or 3' end of the nucleic acid, resulting in the presence of the motif at the N or C terminus of the protein.

The Conjugates

[00199] The cells used in the methods of assaying of the present invention may comprise a conjugate of an arrestin protein and a detectable molecule. In the cells and methods of the present invention, the cells may also comprise a conjugate of a modified GPCR of the present invention and a detectable molecule.

[00200] All forms of arrestin, naturally occurring and engineered variants, including but not limited to, visual arrestin, cone arrestin, βarrestin 1 and βarrestin 2, may be used in the present invention. The modified GPCRs of the present invention may interact to a detectable level with all forms of

arrestin.

[00201] Detectable molecules that may be used to conjugate with the arrestin include, but are not limited to, molecules that are detectable by spectroscopic, photochemical, biochemical, immunochemical, electrical, radioactive, and optical means, including but not limited to bioluminescence, phosphorescence, and fluorescence. Detectable molecules include, but are not limited to, GFP, luciferase, β-galactosidase, rhodamine-conjugated antibody, and the like. Detectable molecules include radioisotopes, epitope tags, affinity labels, enzymes, fluorescent groups, chemiluminescent groups, and the like. Detectable molecules include molecules which are directly or indirectly detected as a function of their interaction with other molecule(s). These detectable molecules should be a biologically compatible molecule and should not compromise the ability of the arrestin to interact with the GPCR system and the interaction of the arrestin with the GPCR system must not compromise the ability of the detectable molecule to be detected. Preferred detectable molecules are optically detectable molecules, including optically detectable proteins, such that they may be excited chemically, mechanically, electrically, or radioactively to emit fluorescence, phosphorescence, or bioluminescence. More preferred detectable molecules are inherently fluorescent molecules, such as fluorescent proteins, including, for example, Green Fluorescent Protein (GFP). The detectable molecule may be conjugated to the arrestin protein by methods as described in Barak et al. (U.S. Patent Nos. 5,891,646 and 6,110,693). The detectable molecule may be conjugated to the arrestin at the front-end, at the back-end, or in the middle.

[00202] The GPCR or biologically active fragments thereof may also be conjugated with a detectable molecule. Preferably, the carboxyl-terminus of the GPCR is conjugated with a detectable molecule. A carboxyl-terminal tail conjugated or attached to a detectable molecule can be used in a carboxyl-terminal tail exchange to provide the detectably labeled GPCR.

[00203] If the GPCR is conjugated with a detectable molecule, proximity of the GPCR with the arrestin may be readily detected. In addition, if the GPCR is conjugated with a detectable molecule, compartmentalization of the GPCR with the arrestin may be readily confirmed. The detectable molecule used to conjugate with the GPCRs may include those as described above, including, for example, optically detectable molecules, such that they may be excited chemically, mechanically, electrically, or radioactively to emit fluorescence, phosphorescence, or bioluminescence. Preferred optically detectable molecules may be detected by immunofluorescence, luminescence, fluorescence, and phosphorescence.

[00204] For example, the GPCRs may be antibody labeled with an antibody conjugated to an immunofluorescence molecule or the GPCRs may be conjugated with a luminescent donor. In particular, the GPCRs may be conjugated with, for example, luciferase, for example, *Renilla* luciferase, or a rhodamine-conjugated antibody, for example, rhodamine-conjugated anti-HA mouse monoclonal antibody. Preferably, the carboxyl-terminal tail of the GPCR may be conjugated with a luminescent donor, for example, luciferase. The GPCR, preferably the carboxyl-terminal tail, also may be conjugated with GFP as described in L. S. Barak et al. "Internal Trafficking and Surface Mobility of a Functionally Intact β_2-Adrenergic Receptor-Green Fluorescent Protein Conjugate", *Mol. Pharm.* (1997) 51, 177 - 184.

<u>**Cell Types and Substrates**</u>

[00205] The cells of the present invention may express at least one modified GPCR of the present invention. The cells may further comprise a conjugate of an arrestin protein and a detectable molecule. Useful cells include eukaryotic and prokaryotic cells, including, but not limited to, bacterial cells, yeast cells, fungal cells, insect cells, nematode cells, plant cells, and animal cells. Suitable animal cells include, but are not limited to, HEK-293 cells, U2OS cells, HeLa cells, COS cells, and various primary

mammalian cells. An animal model expressing a conjugate of an arrestin and a detectable molecule throughout its tissues or within a particular organ or tissue type, may also be used.

[00206] The cells of the present invention may express one modified protein that results in agonist-independent localization of GPCRs to endocytic vesicles or endosomes.

[00207] A substrate may have deposited thereon a plurality of cells of the present invention. The substrate may be any suitable biologically substrate, including but not limited to, glass, plastic, ceramic, semiconductor, silica, fiber optic, diamond, biocompatible monomer, or biocompatible polymer materials.

Methods of detection

[00208] Methods of detecting the intracellular location of the detectably labeled arrestin, the intracellular location of a detectably labeled GPCR, or interaction of the detectably labeled arrestin, or other member of GPCR/arrestin complex with a GPCR or any other cell structure, including for example, the concentration of arrestin or GPCR at a cell membrane, colocalization of arrestin with GPCR in endosomes, and concentration of arrestin or GPCR in clathrin-coated pits, and the like, will vary dependent upon the detectable molecule(s) used.

[00209] One skilled in the art readily will be able to devise detection methods suitable for the detectable molecule(s) used. For optically detectable molecules, any optical method may be used where a change in the fluorescence, bioluminescence, or phosphorescence may be measured due to a redistribution or reorientation of emitted light. Such methods include, for example, polarization microscopy, BRET, FRET, evanescent wave excitation microscopy, and standard or confocal microscopy.

[00210] In a preferred embodiment arrestin may be conjugated to GFP and the arrestin-GFP conjugate may be detected by confocal microscopy.

In another preferred embodiment, arrestin may conjugated to a GFP and the GPCR may be conjugated to an immunofluorescent molecule, and the conjugates may be detected by confocal microscopy. In an additional preferred embodiment, arrestin may conjugated to a GFP and the carboxy-terminus of the GPCR may be conjugated to a luciferase and the conjugates may be detected by bioluminescence resonance emission technology. In a further preferred embodiment arrestin may be conjugated to a luciferase and GPCR may be conjugated to a GFP, and the conjugates may be detected by bioluminescence resonance emission technology. The methods of the present invention are directed to detecting GPCR activity. The methods of the present invention allow enhanced monitoring of the GPCR pathway in real time.

[00211] In a preferred embodiment, the localization pattern of the detectable molecule is determined. In a further preferred embodiment, alterations of the localization pattern of the detectable molecule may be determined. The localization pattern may indicate cellular distribution of the detectable molecule. Certain methods of detection are described in U.S.S.N. 10/095,620, filed March 12, 2002, which claims priority to U.S. Provisional Patent Application No: 60/275,339, filed March 13, 2001, the contents of which are incorporated by reference in their entirety.

[00212] Molecules may also be detected by their interaction with another detectably labeled molecule, such as an antibody.

Test Kits

[00213] In a further embodiment of this invention, commercial test kits including an assay system for screening potential drugs effective to modulate the activity of the GPCR may be prepared. The test kits may include cells, nucleic acids, or proteins described herein. The test kits may be used to carry out any of the methods described herein. A GPCR of interest may be introduced into host cells of the test kit. The test kit may be

useful for determining if the GPCR is expressed at the plasma membrane, if the phosphorylated or unphosphorylated GPCR binds arrestin, or if the phosphorylated or unphosphorylated GPCR is internalized. The test kit may be useful for the identification of compounds that alter the desensitization of the GPCR of interest.

[00214] The GPCR may be introduced into a test system, and the prospective drug may also be introduced into the resulting cell culture, and the culture thereafter examined to observe any changes in the GPCR activity (e.g., signaling, recycling, affinity for arrestin, and the like) in the cells.

[00215] The following examples are presented in order to more fully illustrate the preferred embodiments of the invention. They should in no way be construed, however, as limiting the broad scope of the invention.

EXAMPLES

Example 1

Experimental Procedures

[00216] The present inventors subcloned the Bovine GRK2-C20 cDNA (Inglese et al., Nature 1992) into the expression vector pcDNA3.1zeo+ (Invitrogen). Expression of this cDNA produces GRK2 with a CAAX motif (where C is cysteine, A is a small aliphatic residue, and X is an uncharged amino acid) added to the carboxyl terminus. The specific CAAX motif added to the end of GRK2, CVLL, directs the geranylgeranylation (C20 isoprenylation) of this protein. The enzyme-directed covalent attachment of the 20 carbon geranylgeranyl lipid group to the carboxyl terminus of GRK2 allows it to be localized at the plasma membrane (Inglese et al., Nature 1992).

[00217] Cell Culture

[00218] Human embryonic kidney (HEK-293) cells were purchased from the American Type Culture Collection (ATCC) and grown in Eagle's

minimum essential medium (EMEM) supplemented with 10% (v/v) heat-inactivated fetal calf serum and gentamicin (100 µg/ml). HEK-293 cells stably expressing arrestin-GFP (HEK293-ArrGFP) were generated by standard procedures using G418 selection (0.4 mg/ml). HEK-293 cells were transiently transfected with arrestin-GFP, the GPCR of interest, and GRK2-C20. For control experiments performed in parallel, HEK-293 cells were transiently transfected with arrestin-GFP, the GPCR of interest, and no GRK2-C20. HEK293 cells were transiently transfected with arrestin-GFP, the GPCR of interest and GRK2-C20. For control experiments performed in parallel, the HEK293 cells were transiently transfected with arrestin-GFP were transiently transfected with the GPCR of interest and no GRK2-C20. All transfections were performed by the calcium phosphate coprecipitation method as previously described (Oakley et al., 1999). Following the transfection, cells were maintained in the culture medium (EMEM supplemented with 10% FCS and 10 ug/ml gentamycin) for approximately 24 hours. The cells were then plated on 35 mm glass bottom dishes (MatTek) and incubated for an additional 16-24 hours. Transfected GPCRs included both known GPCRs (receptors for which the natural ligand is know) and orphan GPCRs (receptors for which the natural ligand has not yet been identified).

[00219] Confocal Microscopy

[00220] Transfected HEK-293 cells were plated on 35 mm glass bottom dishes (MatTek) and cultured overnight. The next day, the medium was removed and replaced with serum-free medium supplemented with 10 mM HEPES for an additional 1 hour incubation at 37 °C. The distribution of arrestin-GFP was then assessed using a Zeiss laser scanning confocal microscope (LSM 5 Pascal). Images were acquired with a 63x oil objective from live cells using single line excitation (488 nm) and a LP505 emission filter.

Example 2

Agonist-Independent desensitization of known GPCRs upon expression of a modified GRK

[00221] The present inventors determined that overexpression of the GRK2-C20, which is expressed at the plasma membrane (Inglese et al., Nature 1992), in a cell line expressing arrestin-GFP promoted the binding of arrestin-GFP to GPCRs in the absence of added ligand.

[00222] The HEK293 cells transiently transfected with arrestin-GFP were transiently transfected with the GPCR of interest and with or without GRK2-C20. Using confocal microscopy, the distribution of the arrestin-GFP was determined. The localization of the arrestin-GFP at clathrin coated pits, endocytic vesicles, endosomes, or other stages in the desensitization pathway indicated arrestin-GFP binding to the GPCR. Thus, GPCR desensitization, visualized by the binding of arrestin-GFP to the GPCRs, was analyzed.

[00223] In the absence of added agonist, arrestin-GFP localized in small puncta (presumably clathrin coated pits) at the plasma membrane in cells expressing GRK2-C20 and either the cannabinoid type 2 receptor (CB2R) (Figure 4). Moreover, in the absence of added agonist, arrestin-GFP localized in endocytic vesicles in cells expressing GRK2-C20 and either the angiotensin II type IA receptor (AT1AR), vasopressin V2 receptor (V2R), (Figure 4) or neurokinin-1/substance P receptor (NK-1 or SPR). In control cells expressing each of the receptors (CB2R, AT1AR, V2R, or SPR) but lacking GRK2-C20, arrestin-GFP was diffusely expressed in the cytoplasm and did not localize to any significant extent in pits at the plasma membrane or vesicles inside the cell (Figure 4).

Example 3

Agonist-Independent desensitization of orphan GPCRs upon expression of a modified GRK

[00224] The present inventors determined that overexpression of the GRK2-C20, which is expressed at the plasma membrane (Inglese et al., Nature 1992), in a cell line expressing arrestin-GFP promoted the binding of arrestin-GFP to GPCRs in the absence of added ligand.

[00225] As above, the HEK293 cells transiently transfected with arrestin-GFP were transiently transfected with the GPCR of interest and with or without GRK2-C20. Using confocal microscopy, the distribution of the arrestin-GFP was determined. The localization of the arrestin-GFP at clathrin coated pits, endocytic vesicles, endosomes, or other stages in the desensitization pathway indicated arrestin-GFP binding to the GPCR. Thus, GPCR desensitization, visualized by the binding of arrestin-GFP to the GPCRs, was analyzed.

[00226] In the absence of added agonist, arrestin-GFP localized in small puncta (presumably clathrin coated pits) at the plasma membrane in cells expressing the orphan receptor GPR55 (Figure 7). In control cells expressing GPR55 but lacking GRK2-C20, arrestin-GFP was diffusely expressed in the cytoplasm and did not localize to any significant extent in pits at the plasma membrane or vesicles inside the cell (Figure 7). Other orphan GPCRs are described below.

Example 4

Method of analyzing the ability of a GPCR to bind arrestin

[00227] The present inventors developed a method to determine if a GPCR of interest is expressed at the plasma membrane. Preferably, the expression of orphan GPCRs may be analyzed in host cells in which GPCRs desensitize in an agonist-independent manner, as described herein.

[00228] As above, the HEK293 cells transiently transfected with arrestin-GFP were transiently transfected with the GPCR of interest and with or without GRK2-C20. Using confocal microscopy, the distribution of the arrestin-GFP was determined. The localization of the arrestin-GFP at

clathrin coated pits, endocytic vesicles, endosomes, or other stages in the desensitization pathway indicated arrestin-GFP binding to the GPCR. Thus, GPCR desensitization, visualized by the binding of arrestin-GFP to the GPCRs, was analyzed.

[00229] Certain GPCRs, as described above, localized in clathrin coated pits, endocytic vesicles, endosomes, or other stages in the desensitization pathway. This localization indicated that the GPCRs had the ability to bind arrestin, because arrestin binding is requisite for subsequent localization in the desensitization pathway. A GPCR that does not bind arrestin would not enter or localize in the desensitization pathway. GPCRs that do not bind arrestin may be altered such that they do bind arrestin. The present inventors modified certain GPCRs to enhance arrestin affinity, as described below.

Example 5

Method of increasing the ability of a GPCR to bind arrestin

[00230] The present inventors modified GPCRs to enhance their binding to arrestin. These modifications are described in U.S.S.N. 09/993,844. GPCRs were modified at their C-terminal tails to be better phosphorylated by GRKs. These modified and phosphorylated GPCRs then had enhanced binding to arrestin. They demonstrated increased internalization. The letter E (for enhanced phosphorylation) is added to the end of the name of the GPCR which has been modified in this manner.

[00231] Modified GPCR constructs were generated by polymerase chain reaction following standard protocols and contain the HA epitope. Chimeric receptors were constructed in which the carboxyl-terminal tails of the GPCR and V2R were exchanged (Fig. 3), one for the other, after the putative sites of palmitoylation. Sequences of the DNA constructs were confirmed by DNA sequencing.

[00232] The nucleic acids of the GPCR of interest were PCR-amplified

with primers that introduced a Not I restriction enzyme site (gcggccgc) immediately after the codon for a cysteine residue (a putative site of palmitoylation) 10 to 25 amino acids (preferably 15 to 20) downstream of the NPXXY that is to be fused to the V2R carboxyl terminus. The amplified receptor DNA fragment was then subcloned into the pEArrB-1 vector (described in U.S. Patent Application No. 09/993,844) using the Not I restriction enzyme site and an additional restriction enzyme site upstream of the receptor atg start codon. When expressed, the modified GPCR will contain a 31 amino acid peptide fused to the receptor carboxyl terminus. The first two amino acids will be Ala residues contributed by the Not I site, and the last 29 amino acids will be from the V2R carboxyl terminus.

[00233] The present inventors modified the carboxyl-terminal tails of the following receptors as described above and in the U.S. Patent Application No. 09/993,844: the β2-adrenergic receptor (β2ARE), dopamine D1A receptor (D1ARE), mu opiod receptor (MORE), orphan GPR3 (GPR3E), orphan GPR6 (GPR6E), orphan GPR12 (GPR12E), orphan GPR7 (GPR7E), orphan GPR8 (GPR8E), orphan GPR55 (GPR55E), orphan SREB2 (SREB2E), and orphan SREB3 (SREB3E). The "E" stands for "enhanced arrestin binding". In the absence of added agonist, arrestin-GFP localized in endocytic vesicles for each of the modified GPCRs listed above when co-expressed with GRK2-C20 (Figures 4, 5, 6, and 7). For some of these modified receptors (such as orphan GPR6E), a small but significant amount of arrestin-GFP was observed to localize in intracellular vesicles in the control cells lacking the GRK2-C20 (Figure 5). However, overexpression of GRK2-C20 with these receptors promoted a marked increase in this response (Figure 5). The amino acid and nucleic acid sequences of these modified GPCRs, the wild-type sequences, and sequences of HA-tagged modified GPCRs are shown in Figure 3 and in SEQ ID Nos: 35-90.

Example 6

Method of Determining if a GPCR of interest is expressed at the plasma membrane

[00234] The present inventors developed a method to determine if a GPCR of interest is expressed at the plasma membrane.

[00235] The HEK293 cells transiently transfected with arrestin-GFP were transiently transfected with the GPCR of interest and with or without GRK2-C20. Using confocal microscopy, the distribution of the arrestin-GFP was determined. The localization of the arrestin-GFP at clathrin coated pits, endocytic vesicles, endosomes, or other stages in the desensitization pathway indicated arrestin-GFP binding to the GPCR. Thus, GPCR desensitization, visualized by the binding of arrestin-GFP to the GPCRs, was analyzed.

[00236] Certain GPCRs, as described above, localized in clathrin coated pits, endocytic vesicles, endosomes, or other stages in the desensitization pathway. This localization indicated that the GPCRs were expressed at the plasma membrane, because plasma membrane expression is requisite for subsequent localization in the desensitization pathway. A GPCR that was not expressed at the plasma membrane would not localize in the desensitization pathway. GPCRs that do not express at the plasma membrane may be altered such that they do express at the plasma membrane. For example, the expression of the GPCR may be altered, the amino acid sequence of the GPCR may be altered, or the GPCR may be introduced into another host cell.

Example 7

Monitoring desensitization of GPCR mutants

[00237] Desensitization may be monitored in cells including GPCR mutants. The desensitization of the GPCR mutant may be dependent on GRK overexpression.

[00238] A vector including the human β_2AR-E-Y326A containing a point

mutation, the Tyrosine residue 326 converted to Alanine, will be transfected into cells expressing arrestin-GFP and a GRK, which may be modified. The "E" indicates that the GPCR has been modified, as described above. The Y326A mutation causes the GPCR to be dependent on overexpressed GRK for phosphorylation and subsequent desensitization. The β_2AR-Y326A will desensitize in the absence of agonist upon expression of GRK-C20. The expression of the GRK may be altered, including methods of altering the amount of GRK nucleic acids in the cell using an inducible promoter, replication controlling machinery such as the origin of replication, or manually altering the amount of vector in the cells.

[00239] The cells will be seeded in 96 well or higher density plates and incubated overnight. The next morning the activator of the inducible system or vehicle only will be added to the wells to induce overexpression of the GRK or modified GRK. Agonist will be added to cells expressing the GRK (not the modified GRK).

[00240] Compounds of interest will then be added to the wells to see if they alter the internalization of arrestin-GFP. The cells will then be fixed with 2% paraformaldehyde and the amount of arrestin-GFP translocation will be measured using image analysis systems.

[00241] While the invention has been described and illustrated herein by references to various specific material, procedures and examples, it is understood that the invention is not restricted to the particular material combinations of material, and procedures selected for that purpose. Numerous variations of such details can be implied as will be appreciated by those skilled in the art.

[00242] The following is a list of documents related to the above disclosure and particularly to the experimental procedures and discussions. The following documents, as well as any documents referenced in the

foregoing text, should be considered as incorporated by reference in their entirety.

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CLAIMS

1. A method of identifying a compound which alters GPCR internalization, comprising:

(a) providing a cell comprising a GPCR, an arrestin, and a modified GRK, wherein said GPCR is at least partially internalized in an agonist-independent manner upon expression of said GRK;

(b) exposing said cell to the compound(s);

(c) determining the cellular distribution of the GPCR, arrestin, or modified GRK; and

(d) monitoring a difference between (1) the distribution of the GPCR, arrestin, or modified GRK in the cell in the presence of the compound(s) and (2) the distribution of the GPCR, arrestin, or modified GRK in the cell in the absence of the compound(s).

2. The method of claim 1, wherein the GRK of step (a) is overexpressed.

3. The method of claim 1, wherein the expression of the GRK of step (a) is inducible.

4. The method of claim 1, wherein the GRK comprises a CAAX motif.

5. The method of claim 1, wherein the GPCR is modified to have enhanced phosphorylation by a GRK.

6. The method of claim 1, wherein the GPCR is β_2AR(Y326A).

7. The method of claim 1, wherein the GPCR is a GPCR listed in Figure 1, an orphan GPCR, a modified GPCR, a taste receptor, a Class A

GPCR, a Class B GPCR, a mutant GPCR, or a biologically active fragment thereof.

8. The method of claim 1, wherein the GRK is GRK1, GRK2, GRK3, GRK4, GRK5, GRK6, or a biologically active fragment thereof.

9. The method of claim 1, wherein the GPCR, GRK, or arrestin is detectably labeled.

10. The method of claim 1, wherein a molecule involved in desensitization is detectably labeled, or a molecule that interacts with a molecule involved in desensitization is detectably labeled.

11. The method of claim 1, wherein the arrestin is visual arrestin, cone arrestin, β-arrestin 1, β-arrestin 2, or a biologically active fragment thereof.

12. The method of claim 1, wherein an agonist is not provided.

13. The method of claim 1, wherein a difference between (1) and (2) of step (d) indicates modulation of GPCR internalization.

14. A method of identifying a compound that alters GPCR phosphorylation, comprising:

(a) providing a cell comprising a GPCR and a GRK;

(b) exposing said cell to the compound(s); and

(c) determining whether GRK phosphorylation of the GPCR is altered in the presence of the compound(s).

15. The method of claim 14, wherein the cellular distribution of the

GPCR or GRK is determined.

16. The method of claim 14, wherein a difference between (1) the distribution of the GPCR or GRK in the cell in the presence of the compound and (2) the distribution of the GPCR or GRK in the cell in the absence of the compound(s) is monitored.

17. The method of claim 16, wherein a difference is correlated between (1) and (2) to the phosphorylation of the GPCR.

18. The method of claim 14, wherein the GRK is not located in the plasma membrane, indicating that GRK phosphorylation of the GPCR is altered.

19. The method of claim 14, wherein the phosphorylation state of the GPCR is determined.

20. The method of claim 14, wherein the activity of the GRK is determined.

21. The method of claim 17, wherein the ability of the GPCR to be internalized is determined.

22. A method of determining if a GPCR of interest is expressed at the plasma membrane, comprising:

(a) providing a cell comprising a GPCR, an arrestin, and a GRK, wherein the arrestin is detectably labeled;

(b) determining the cellular distribution of the arrestin; and

(c) correlating the cellular distribution of the arrestin to the ability of the GPCR to be expressed at the plasma membrane.

23. The method of claim 22, wherein the arrestin is localized in vesicles, pits, endosomes, or elsewhere in the desensitization pathway.

24. A method of determining if a GPCR of interest is expressed at the plasma membrane, comprising:

(a) providing a cell comprising a GPCR and a GRK, wherein the GRK is detectably labeled;

(b) determining the cellular distribution of the GRK; and

(c) correlating the cellular distribution of the GRK to the ability of the GPCR to be expressed at the plasma membrane.

25. The method of claim 24, wherein the GRK is localized at the plasma membrane.

26. A method of analyzing the ability of a GPCR to bind arrestin, comprising:

(a) providing a cell comprising a GPCR, an arrestin, and a GRK, wherein the arrestin is detectably labeled;

(b) determining the cellular distribution of the arrestin; and

(c) correlating the cellular distribution of the arrestin to the ability of the GPCR to bind arrestin.

27. The method of claim 26, wherein the arrestin or the GPCR is localized in vesicles, pits, or endosomes.

28. A compound identified by claim 1.

29. A method of treating a disease by modulating desensitization of a GPCR in a host cell, comprising:

(a) providing a compound identified in claim 1; and

(b) administering said compound to a host.

30. A host cell comprising a GPCR and a modified GRK.

31. The host cell of claim 30, wherein the GRK is inducible or over-expressed.

32. The host cell of claim 30, wherein said host cell further comprises arrestin, wherein said arrestin may be detectably labeled.

33. The host cell of claim 30, wherein at least one of the GPCR, GRK, another molecule involved in desensitization, or a molecule that interacts with a molecule involved in desensitization is detectably labeled.

34. A method of modifying a nucleic acid encoding a GRK in which a GPCR is constitutively internalized, comprising:

(a) providing a nucleic acid encoding a GRK;

(b) mutating the nucleic acid encoding a GRK such that the encoded GRK comprises a CAAX motif, wherein said modified GRK phosphorylates a GPCR in the absence of agonist; and

(c) expressing the modified GRK in a cell.

35. The method of claim 34, wherein the nucleic acid encoding a GRK comprises SEQ ID No: 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, or 34.

36. A kit for identifying a compound that modulates the internalization of a GPCR, comprising the host cell of claim 30.

37. A modified GPCR comprising a NPXXY motif, and a carboxyl

terminal tail,

wherein said carboxyl terminal tail comprises a putative site of palmitoylation and one or more clusters of phosphorylation,

wherein the carboxyl terminal tail comprises a retained portion of a carboxyl-terminus region of a first GPCR portion fused to a portion of a carboxyl-terminus from a second GPCR, and

wherein the second GPCR comprises the one or more clusters of phosphorylation and further comprises a second putative site of palmitoylation approximately 10 to 25 amino acid residues downstream of a second NPXXY motif.

38. The modified GPCR of claim 37, wherein the first GPCR is a Class A receptor.

39. The modified GPCR of claim 37, wherein the first GPCR is hGPR3, hGPR6, hGPR12, hSREB2, hSREB3, hGPR8, or hGPR22.

40. The modified GPCR of claim 37, wherein the second GPCR is a Class B receptor.

41. The modified GPCR of claim 37, wherein the Class B receptor is selected from the group consisting of a vasopressin V2 receptor, a neurotensin-1 receptor, a substance P receptor and an oxytocin receptor.

42. A nucleic acid encoding a modified GPCR of claim 37.

43. A nucleic acid selected from the group consisting of SEQ ID Nos: 36, 38, 40, 42, 44, 46, 48, 50, 52, 54, 56, 58, 60, 62, 64, 66, 68, 70, 72, 74, 76, 78, 80, 82, 84, 86, 88, and 90.

44. An expression vector comprising the nucleic acid of claim 42.

45. A host cell comprising the expression vector of claim 44.

46. A host cell comprising the nucleic acid of claim 42.

47. A method of screening compounds for GPCR activity comprising the steps of:

(a) providing a cell that expresses at least one modified GPCR according to claim 37, wherein said cell further comprises arrestin conjugated to a detectable molecule;

(b) exposing the cell to the compound;

(c) detecting location of the arrestin within the cell;

(d) comparing the location of the arrestin within the cell in the presence of the compound to the location of the arrestin within the cell in the absence of the compound; and

(e) correlating a difference between (1) the location of the arrestin within the cell in the presence of the compound and (2) the location of the arrestin within the cell in the absence of the compound.

48. The method of Claim 47, wherein the arrestin is detected in endosomes, endocytic vesicles, or pits.

49. A kit for identifying a molecule that modulates the activity of a GPCR, comprising a cell that expresses at least one modified GPCR according to claim 37, wherein said cell further comprises a molecule involved in desensitization conjugated to a detectable molecule.

FIG. 1

Human G Protein Coupled Receptor Family

(Receptors known as of January, 1999)

CLASS	LIGAND	NUMBER	TISSUE	PHYSIOLOGY	THERAPEUTICS
•Class I					
Rhodopsin like					
	•Amine				
	·Acetylcholine (muscarinic & nicotinic)	5	Brain, Nerves, Heart	Neurotransmitter	Acuity, Alzheimer's
	·Adrenoceptors				
	·Alpha Adrenoceptors	6	Brain, Kidney, Lung	Gluconeogenesis	Diabetes, Cardiovascular
	·Beta Adrenoceptors	3	Kidney, Heart	Muscle Contraction	Cardiovascular, Respiratory
	·Dopamine	5	Brain, Kidney, GI	Neurotransmitter	Cardiovascular, Parkinson's
	·Histamine	2	Vascular, Heart, Brain	Vascular Permeability	Anti-inflammatory, Ulcers
	·Serotonin (5-HT)	16	Most Tissues	Neurotransmitter	Depression, Insomnia, Analgesic
	•Peptide				
	·Angiotensin	2	Vascular, Liver, Kidney	Vasoconstriction	Cardiovascular, Endocrine
	·Bradykinin	1	Liver, Blood	Vasodilation,	Anti-inflammatory, Asthma
	·C5a anaphylatoxin	1	Blood	Immune System	Anti-inflammatory
	·Fmet-leu-phe	3	Blood	Chemoattractant	Anti-inflammatory
	·Interleukin-8	1	Blood	Chemoattractant	Anti-inflammatory
	·Chemokine	6	Blood	Chemoattractant	Anti-inflammatory
	·Orexin	2	Brain	Fat Metabolism	Obesity
	·Nociceptin	1	Brain	Bronchodilator, Pain	Airway Diseases, Anesthetic
	·CCK (Gastrin)	2	Gastrointestinal	Motility, Fat Absorption	Gastrointestinal, Obesity, Parkinson's
	·Endothelin	2	Heart, Bronchus, Brain	Muscle Contraction	Cardiovascular, Respiratory
	·Melanocortin	5	Kidney, Brain	Metabolic Regulation	Anti-inflammatory, Analgesics
	·Neuropeptide Y	5	Nerves, Intestine, Blood	Neurotransmitter	Behavior, Memory, Cardio-vascular
	·Neurotensin	1	Brain,	CNS	Cardiovascular, Analgesic

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·Opioid	3	Brain,	CNS	Depression, Analgesic
·Somatostatin	5	Brain, Gastrointestinal	Neurotransmitter	Oncology, Alzheimer's
·Tachykinin				
(Substance P, NKA_1)	3	Brain Nerves	Neurohormone	Depression, Analgesic
·Thrombin	3	Platelets, Blood Vessels	Coagulation	Anti-coagulant, Anti-inflammatory
·Vasopressin-like	4	Arteries, Heart, Bladder	Water Balance	Anti-diuretic, Diabetic Complications
·Galanin	1	Brain, Pancreas	Neurotransmitter	Analgesics, Alzheimer's
·Hormone protein				
·Follicle stimulating hormone	1	Ovary, Testis	Endocrine	Infertility
·Lutropin-choriogonadotropic	1	Ovary, Testis	Endocrine	Infertility
·Thyrotropin	1	Thyroid	Endocrine	Thyroidism, Metabolism
·(Rhod)opsin				
·Opsin	5	Eye	Photoreception	Ophthalmic Diseases
·Olfactory	4(~1000)	Nose	Smell	Olfactory Diseases
·Prostanoid				
·Prostaglandin	5	Arterial, Gastrointestinal	Vasodilation, Pain	Cardiovascular, Analgesic
·Lysophosphatidic Acid	2	Vessels, Heart, Lung	Inflammation	Cancer, Anti-Inflammatory
·Sphingosine-1-phosphate	2	Most Cells	Cell proliferation	Cancer
·Leukotriene	1	White Blood Cells, Bronchus	Inflammation	Asthma, Rheumatoid Arthritis
·Prostacyclin	1	Arterial, Gastrointestinal	Platelet Regulation	Cardiovascular
·Thromboxane	1	Arterial, Bronchus	Vasoconstriction	Cardiovascular, Respiratory
·Nucleotide-like				
·Adenosine	4	Vascular, Bronchus	Multiple Effects	Cardiovascular, Respiratory
·Purinoceptors	4	Vascular, Platelets	Relaxes Muscle	Cardiovascular, Respiratory
·Cannabis	2	Brain	Sensory Perception	Analgesics, Memory
·Platelet activating factor	1	Most Peripheral Tissues	Inflammation	Anti-inflammatory, Anti-asthmatic

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	·Gonadotropin-releasing hormone like				
	·Gonadotropin-releasing hormone	1	Reproductive Organs, Pituitary	Reproduction	Prostate Cancer, Endometriosis
	·Thyrotropin-releasing hormone	1	Pituitary, Brain	Thyroid Regulation	Metabolic Regulation
	·Growth hormone- inhibiting factor	1	Gastrointestinal	Neuroendocrine	Oncology, Alzheimer's
	·Melatonin	1	Brain, Eye, Pituitary	Neuroendocrine	Regulation of Circadian Cycle
•Class II Secretin like					
	·Secretin	1	Gastrointestinal, Heart	Digestion	Obesity, Gastrointestinal
	·Calcitonin	1	Bone, Brain	Calcium Resorption	Osteoporosis
	·Corticotropin releasing factor/urocortin	1	Adrenal, Vascular, Brain	Neuroendocrine	Stress, Mood, Obesity
	·Gastric inhibitory peptide (GIP)	1	Adrenals, Fat Cells	Sugar/Fat Metabolism	Diabetes, Obesity
	·Glucagon	1	Liver, Fat Cells, Heart	Gluconeogenesis	Cardiovascular
	·Glucagon-like Peptide 1 (GLP-1)	1	Pancreas, Stomach, Lung	Gluconeogenesis	Cardiovascular, Diabetes, Obesity
	·Growth hormone-releasing hormone		Brain	Neuroendocrine	Growth Regulation
	·Parathyroid hormone	1	Bone, Kidney	Calcium Regulation	Osteoporosis
	·PACAP	1	Brain, Pancreas, Adrenals	Metabolism	Metabolic Regulation
	·Vasoactive intestinal polypeptide (VIP)	1	Gastrointestinal	Motility	Gastrointestinal
•Class III					
	·Metabotropic Glutamate	7	Brain	Sensory Perception	Hearing, Vision
	·$GABA_B$	1	Brain	Neurotransmitter	Mood Disorders
	·Extracellular Calcium Sensing	1	Parathyroid, Kidney, GI Tract	Calcium Regulation	Cataracts, GI Tumors

Fig. 1, pg. 3 of 3

Fig. 2

Bovine GRK2-C20 Amino Acid Sequence

MADLEAVLADVSYLMAMEKSKATPAARASKKILLPEPSIRSVMQKYLEDRGEVTFEKIFSQKLGYLLFRDFCLKHLEEAK
PLVEFYEEIKKYEKLETEEERLVCSREIFDTYIMKELLACSHPFSKSAIEHVQGHLVKKQVPPDLFQPYIEEICQNLRGD
VFQKFIESDKFTRFCQWKNVELNIHLTMNDFSVHRIIGRGGFGEVYGCRKADTGKMYAMKCLDKKRIKMKQGETLALNER
IMLSLVSTGDCPFIVCMSYAFHTPDKLSFILDLMNGGDLHYHLSQHGVFSEADMRFYAAEIILGLEHMHNRFVVYRDLKP
ANILLDEHGHVRISDLGLACDFSKKKPHASVGTHGYMAPEVLQKGVAYDSSADWFSLGCMLFKLLRGHSPFRQHKTKDKH
EIDRMTLTMAVELPDSFSPELRSLLEGLLQRDVNRRLGCLGRGAQEVKESPFFRSLDWQMVFLQKYPPPLIPPRGEVNAA
DAFDIGSFDEEDTKGIKLLDSDQELYRNFPLTISERWQQEVAETVFDTINAETDRLEARKKTKNKQLGHEEDYALGKDCI
MHGYMSKMGNPFLTQWQRRYFYLFPNRLEWRGEGEAPQSLLTMEEIQSVEETQIKERKCLLLKIRGGKQFVLQCDSDPEL
VQWKKELRDAYREAQQLVQRVPKMKNKPRSPVVELSKVPLIQRGSCVLL

SEQ ID NO: 1

Bovine GRK2-C20 Nucleotide sequence

ATGGCGGACCTGGAGGCGGTGCTGGCCGACGTGAGCTACCTGATGGCCATGGAGAAGAGCAAGGCCACGCCGGCGGCGCG
CGCCAGCAAGAAGATCCTGCTGCCCGAGCCCAGCATCCGCAGCGTCATGCAGAAGTACCTGGAGGACCGGGGCGAGGTGA
CTTTTGAGAAGATCTTCTCCCAGAAGCTGGGGTACCTGCTTTTCCGAGACTTCTGCCTGAAGCACCTGGAGGAGGCCAAG
CCCTTGGTAGAGTTCTACGAGGAGATCAAGAAATACGAGAAGCTGGAGACAGAGGAGGAGCGCCTGGTCTGCAGCCGAGA
GATCTTCGACACGTACATCATGAAGGAGCTGCTGGCCTGCTCACATCCTTTCTCGAAGAGCGCCATTGAGCACGTCCAGG
GCCATCTGGTGAAGAAGCAGGTGCCTCCGGATCTCTTCCAGCCATATATTGAAGAAATTTGCCAGAACCTCCGAGGAGAC
GTGTTCCAGAAATTCATCGAGAGCGATAAATTCACACGATTTTGCCAGTGGAAGAATGTAGAGCTCAACATCCACCTGAC
CATGAACGACTTCAGTGTGCACCGCATCATCGGGCGAGGCGGCTTCGGTGAGGTCTACGGCTGCCGGAAGGCCGACACGG
GCAAGATGTACGCCATGAAGTGTCTGGACAAGAAGCGCATCAAGATGAAGCAAGGGGAGACTCTGGCCCTGAATGAGCGC
ATCATGCTGTCGCTCGTCAGCACCGGGGACTGCCCGTTCATCGTCTGCATGTCATACGCCTTCCACACACCGGACAAGCT
CAGCTTCATCCTGGATCTCATGAACGGCGGGGACCTGCACTACCACCTGTCCCAGCACGGGGTCTTCTCCGAGGCCGACA
TGCGTTTCTACGCCGCCGAGATCATCCTGGGCCTGGAGCACATGCACAACCGCTTCGTGGTCTACCGGGACCTGAAGCCG
GCCAACATCCTGCTGGACGAGCACGGCCACGTGCGCATCTCAGACCTGGGCCTGGCCTGTGACTTCTCCAAGAAGAAGCC
TCACGCCAGTGTGGGCACCCACGGGTACATGGCTCCCGAGGTTCTACAGAAGGGTGTGGCCTACGACAGCAGCGCCGACT
GGTTCTCCCTGGGCTGCATGCTCTTCAAGCTGCTGCGAGGGCATAGCCCTTTCCGGCAGCACAAGACCAAAGACAAGCAT
GAGATCGACAGAATGACATTGACAATGGCTGTGGAGCTGCCTGACTCCTTCTCCCCTGAGCTCCGCTCCTTGCTGGAGGG
GCTGCTGCAGAGGGATGTCAACCGGAGGCTAGGCTGCCTGGGCCGAGGGGCCCAGGAGGTGAAGGAGAGCCCCTTCTTCC
GTTCCCTGGACTGGCAGATGGTCTTTTTACAAAAGTACCCTCCCCCGTTGATCCCCCCACGAGGGGAGGTGAATGCAGCC
GACGCCTTTGACATTGGCTCCTTCGATGAGGAGGACACAAAAGGAATCAAGCTACTGGACAGTGACCAGGAGCTCTACCG
CAACTTCCCCCTGACCATCTCGGAGCGGTGGCAGCAGGAGGTAGCAGAGACTGTCTTTGACACCATCAATGCTGAGACGG
ACCGGCTGGAGGCCCGCAAGAAAACCAAAAACAAGCAGTTGGGCCACGAGGAAGACTACGCCCTGGGCAAGGACTGCATC
ATGCATGGCTACATGTCCAAGATGGGCAACCCCTTCCTGACCCAGTGGCAGCGGCGGTACTTCTACCTGTTCCCTAACCG
GCTCGAGTGGCGGGGCGAGGGCGAGGCCCCGCAGAGCCTGCTGACCATGGAGGAGATCCAGTCGGTGGAGGAGACGCAGA
TCAAGGAGCGAAAGTGCCTCCTCCTCAAGATCCGAGGTGGCAAGCAGTTTGTCCTGCAGTGCGATAGTGACCCAGAGCTG
GTGCAGTGGAAGAAGGAGCTTCGAGACGCCTACCGCGAGGCCCAGCAGCTAGTGCAGCGGGTGCCCAAGATGAAGAACAA
GCCGCGCTCGCCCGTCGTGGAGCTGAGCAAGGTGCCACTGATCCAGCGCGGCAGTTGTGTGCTTCTTTAG

SEQ ID NO: 2

Amino acid sequence of the GRK2 (G protein-coupled receptor kinase 2 [Homo Sapiens]) Accession NP_001610 (Also called: Adrenergic, beta, receptor kinase 1(ADRBK1)
(Beta-adrenergic receptor kinase 1 (Bark-1))

MADLEAVLADVSYLMAMEKSKATPAARASKKILLPEPSIRSVMQKYLEDRGEVTFEKIFSQKLGYLLFRDFCLNHLEEAR
PLVEFYEEIKKYEKLETEEERVARSREIFDSYIMKELLACSHPFSKSATEHVQGHLGKKQVPPDLFQPYIEEICQNLRGD
VFQKFIESDKFTRFCQWKNVELNIHLTMNDFSVHRIIGRGGFGEVYGCRKADTGKMYAMKCLDKKRIKMKQGETLALNER
IMLSLVSTGDCPFIVCMSYAFHTPDKLSFILDLMNGGDLHYHLSQHGVFSEADMRFYAAEIILGLEHMHNRFVVYRDLKP
ANILLDEHGHVRISDLGLACDFSKKKPHASVGTHGYMAPEVLQKGVAYDSSADWFSLGCMLFKLLRGHSPFRQHKTKDKH
EIDRMTLTMAVELPDSFSPELRSLLEGLLQRDVNRRLGCLGRGAQEVKESPFFRSLDWQMVFLQKYPPPLIPPRGEVNAA
DAFDIGSFDEEDTKGIKLLDSDQELYRNFPLTISERWQQEVAETVFDTINAETDRLEARKKAKNKQLGHEEDYALGKDCI
MHGYMSKMGNPFLTQWQRRYFYLFPNRLEWRGEGEAPQSLLTMEEIQSVEETQIKERKCLLLKIRGGKQFILQCDSDPEL
VQWKKELRDAYREAQQLVQRVPKMKNKPRSPVVELSKVPLVQRGSANGL

SEQ ID NO: 3

Nucleotide sequence for the GRK2 G protein-coupled receptor kinase 2 [Homo Sapiens] Accession NM_001619 (Also called: Adrenergic, beta, receptor kinase 1(ADRBK1) (Beta-adrenergic receptor kinase 1 (Bark-1))

ATGGCGGACCTGGAGGCGGTGCTGGCCGACGTGAGCTACCTGATGGCCATGGAGAAGAGCAAGGCCACGCCGGCCGCGCGCGCCAGCAA
GAAGATACTGCTGCCCGAGCCCAGCATCCGCAGTGTCATGCAGAAGTACCTGGAGGACCGGGGCGAGGTGACCTTTGAGAAGATCTTTT
CCCAGAAGCTGGGGTACCTGCTCTTCCGAGACTTCTGCCTGAACCACCTGGAGGAGGCCAGGCCCTTGGTGGAATTCTATGAGGAGATC
AAGAAGTACGAGAAGCTGGAGACGGAGGAGGAGCGTGTGGCCCGCAGCCGGGAGATCTTCGACTCATACATCATGAAGGAGCTGCTGGC
CTGCTCGCATCCCTTCTCGAAGAGTGCCACTGAGCATGTCCAAGGCCACCTGGGGAAGAAGCAGGTGCCTCCGGATCTCTTCCAGCCAT
ACATCGAAGAGATTTGTCAAAACCTCCGAGGGGACGTGTTCCAGAAATTCATTGAGAGCGATAAGTTCACACGGTTTTGCCAGTGGAAG
AATGTGGAGCTCAACATCCACCTGACCATGAATGACTTCAGCGTGCATCGCATCATTGGGCGCGGGGGCTTTGGCGAGGTCTATGGGTG
CCGGAAGGCTGACACAGGCAAGATGTACGCCATGAAGTGCCTGGACAAAAAGCGCATCAAGATGAAGCAGGGGGAGACCCTGGCCCTGA
ACGAGCGCATCATGCTCTCGCTCGTCAGCACTGGGGACTGCCCATTCATTGTCTGCATGTCATACGCGTTCCACACGCCAGACAAGCTC
AGCTTCATCCTGGACCTCATGAACGGTGGGGACCTGCACTACCACCTCTCCCAGCACGGGGTCTTCTCAGAGGCTGACATGCGCTTCTA
TGCGGCCGAGATCATCCTGGGCCTGGAGCACATGCACAACCGCTTCGTGGTCTACCGGGACCTGAAGCCAGCCAACATCCTTCTGGACG
AGCATGGCCACGTGCGGATCTCGGACCTGGGCCTGGCCTGTGACTTCTCCAAGAAGAAGCCCCATGCCAGCGTGGGCACCCACGGGTAC
ATGGCTCCGGAGGTCCTGCAGAAGGGCGTGGCCTACGACAGCAGTGCCGACTGGTTCTCTCTGGGGTGCATGCTCTTCAAGTTGCTGCG
GGGGCACAGCCCCTTCCGGCAGCACAAGACCAAAGACAAGCATGAGATCGACCGCATGACGCTGACGATGGCCGTGGAGCTGCCCGACT
CCTTCTCCCCTGAACTACGCTCCCTGCTGGAGGGGTTGCTGCAGAGGGATGTCAACCGGAGATTGGGCTGCCTGGGCCGAGGGGCTCAG
GAGGTGAAAGAGAGCCCCTTTTTCCGCTCCCTGGACTGGCAGATGGTCTTCTTGCAGAAGTACCCTCCCCCGCTGATCCCCCCACGAGG
GGAGGTGAACGCGGCCGACGCCTTCGACATTGGCTCCTTCGATGAGGAGGACACAAAAGGAATCAAGTTACTGGACAGTGATCAGGAGC
TCTACCGCAACTTCCCCCTCACCATCTCGGAGCGGTGGCAGCAGGAGGTGGCAGAGACTGTCTTCGACACCATCAACGCTGAGACAGAC
CGGCTGGAGGCTCGCAAGAAAGCCAAGAACAAGCAGCTGGGCCATGAGGAAGACTACGCCCTGGGCAAGGACTGCATCATGCATGGCTA
CATGTCCAAGATGGGCAACCCCTTCCTGACCCAGTGGCAGCGGCGGTACTTCTACCTGTTCCCCAACCGCCTCGAGTGGCGGGGCGAGG
GCGAGGCCCCGCAGAGCCTGCTGACCATGGAGGAGATCCAGTCGGTGGAGGAGACGCAGATCAAGGAGCGCAAGTGCCTGCTCCTCAAG
ATCCGCGGTGGGAAACAGTTCATTTTGCAGTGCGATAGCGACCCTGAGCTGGTGCAGTGGAAGAAGGAGCTGCGCGACGCCTACCGCGA
GGCCCAGCAGCTGGTGCAGCGGGTGCCCAAGATGAAGAACAAGCCGCGCTCGCCCGTGGTGGAGCTGAGCAAGGTGCCGCTGGTCCAGC
GCGGCAGTGCCAACGGCCTCTGA

SEQ ID NO: 4

GRK6 Splice Variant B (AF040751):

MELENIVANTVLLKAREGGGGNRKGKSKKWRQMLQFPHISQCEELRLSLERDYHSLCERQPIGRLLFREFCATRPELSRCVAFLDGVAE
YEVTPDDKRKACGRQLTQNFLSHTGPDLIPEVPRQLVTNCTQRLEQGPCKDLFQELTRLTHEYLSVAPFADYLDSIYFNRFLQWKWLER
QPVTKNTFRQYRVLGKGGFGEVCACQVRATGKMYACKKLEKKRIKKRKGEAMALNEKQILEKVNSRFVVSLAYAYETKDALCLVLTLMN
GGDLKFHIYHMGQAGFPEARAVFYAAEICCGLEDLHRERIVYRDLKPENILLDDHGHIRISDLGLAVHVPEGQTIKGRVGTVGYMAPEV
VKNERYTFSPDWWALGCLLYEMIAGQSPFQQRKKKIKREEVERLVKEVPEEYSERFSPQARSLCSQLLCKDPAERLGCRGGSAREVKEH
PLFKKLNFKRLGAGMLEPPFKPDPQAIYCKDVLDIEQFSTVKGVELEPTDQDFYQKFATGSVPIPWQNEMVETECFQELNVFGLDGSVP
PDLDWKGQPPAPPKKGLLQRLFSRQRIAVETAATARKSSPPASSPQPEAPTSSWR

SEQ ID NO: 5

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GCGGCAGATGCTCCAGTTCCCTCACATCAGCCAGTGCGAAGAGCTGCGGCTCAGCCTCGAGCGTGACTATCACAGCCTGTGCGAGCGGC
AGCCCATTGGGCGCCTGCTGTTCCGAGAGTTCTGTGCCACGAGGCCGGAGCTGAGCCGCTGCGTCGCCTTCCTGGATGGGGTGGCCGAG
TATGAAGTGACCCCGGATGACAAGCGGAAGGCATGTGGGCGGCAGCTAACGCAGAATTTTCTGAGCCACACGGGTCCTGACCTCATCCC
TGAGGTCCCCCGGCAGCTGGTGACGAACTGCACCCAGCGGCTGGAGCAGGGTCCCTGCAAAGACCTTTTCCAGGAACTCACCCGGCTGA
CCCACGAGTACCTGAGCGTGGCCCCTTTTGCCGACTACCTCGACAGCATCTACTTCAACCGTTTCCTGCAGTGGAAGTGGCTGGAAAGG
CAGCCAGTGACCAAAAACACCTTCAGGCAATACCGAGTCCTGGGCAAAGGTGGCTTTGGGGAGGTGTGCGCCTGCCAGGT
GCGGGCCACAGGTAAGATGTATGCCTGCAAGAAGCTAGAGAAAAAGCGGATCAAGAAGCGGAAAGGGGAGGCCATGGCGCTGAACGAGA
AGCAGATCCTGGAGAAAGTGAACAGTAGGTTTGTAGTGAGCTTGGCCTACGCCTATGAGACCAAGGACGCGCTGTGCCTGGTGCTGACA
CTGATGAACGGGGGCGACCTCAAGTTCCACATCTACCACATGGGCCAGGCTGGCTTCCCCGAAGCGCGGGCCGTCTTCTACGCCGCCGA
GATCTGCTGTGGCCTGGAGGACCTGCACCGGGAGCGCATCGTGTACAGGGACCTGAAGCCCGAGAACATCTTGCTGGATGACCACGGCC
ACATCCGCATCTCTGACCTGGGACTAGCTGTGCATGTGCCCGAGGGCCAGACCATCAAAGGGCGTGTGGGCACCGTGGGTTACATGGCT
CCGGAGGTGGTGAAGAATGAACGGTACACGTTCAGCCCTGACTGGTGGGCGCTCGGCTGCCTCCTGTACGAGATGATCGCAGGCCAGTC
GCCCTTCCAGCAGAGGAAGAAGAAGATCAAGCGGGAGGAGGTGGAGCGGCTGGTGAAGGAGGTCCCCGAGGAGTATTCCGAGCGCTTTT
CCCCGCAGGCCCGCTCACTTTGCTCACAGCTCCTCTGCAAGGACCCTGCCGAACGCCTGGGGTGTCGTGGGGGCAGTGCCCGCGAGGTG
AAGGAGCACCCCCTCTTTAAGAAGCTGAACTTCAAGCGGCTGGGAGCTGGCATGCTGGAGCCGCCGTTCAAGCCTGACCCCCAGGCCAT
TTACTGCAAGGATGTTCTGGACATTGAACAGTTCTCTACGGTCAAGGGCGTGGAGCTGGAGCCTACCGACCAGGACTTCTACCAGAAGT
TTGCCACAGGCAGTGTGCCCATCCCCTGGCAGAACGAGATGGTGGAGACCGAGTGCTTCCAAGAGCTGAATGTCTTTGGGCTGGATGGC
TCAGTTCCCCCAGACCTGGACTGGAAGGGCCAGCCACCTGCACCTCCTAAAAAGGGACTGCTGCAGAGACTCTTCAGTCGCCAAAGGAT
TGCTGTGGAAACTGCAGCGACAGCGAGGAAGAGCTCCCCACCCGCCTCTAGCCCCCAGCCCGAGGCCCCCACCAGCAGTTGGCGGTAG

SEQ ID NO: 6

Human GRK6 Splice Variant C (AF040752):

MELENIVANTVLLKAREGGGGNRKGKSKKWRQMLQFPHISQCEELRLSLERDYHSLCERQPIGRLLFREFCATRPELSRCVAFLDGVAE
YEVTPDDKRKACGRQLTQNFLSHTGPDLIPEVPRQLVTNCTQRLEQGPCKDLFQELTRLTHEYLSVAPFADYLDSIYFNRFLQWKWLER
QPVTKNTFRQYRVLGKGGFGEVCACQVRATGKMYACKKLEKKRIKKRKGEAMALNEKQILEKVNSRFVVSLAYAYETKDALCLVLTLMN
GGDLKFHIYHMGQAGFPEARAVFYAAEICCGLEDLHRERIVYRDLKPENILLDDHGHIRISDLGLAVHVPEGQTIKGRVGTVGYMAPEV
VKNERYTFSPDWWALGCLLYEMIAGQSPFQQRKKKIKREEVERLVKEVPEEYSERFSPQARSLCSQLLCKDPAERLGCRGGSAREVKEH
PLFKKLNFKRLGAGMLEPPFKPDPQAIYCKDVLDIEQFSTVKGVELEPTDQDFYQKFATGSVPIPWQNEMVETECFQELNVFGLDGSVP
PDLDWKGQPPAPPKKGLLQRLFSRQR

SEQ ID NO: 7

ATGGAGCTCGAGAACATCGTAGCGAACACGGTGCTACTCAAGGCCCGGGAAGGTGGCGGTGGAAATCGCAAAGGCAAAAGCAAGAAATG
GCGGCAGATGCTCCAGTTCCCTCACATCAGCCAGTGCGAAGAGCTGCGGCTCAGCCTCGAGCGTGACTATCACAGCCTGTGCGAGCGGC
AGCCCATTGGGCGCCTGCTGTTCCGAGAGTTCTGTGCCACGAGGCCGGAGCTGAGCCGCTGCGTCGCCTTCCTGGATGGGGTGGCCGAG
TATGAAGTGACCCCGGATGACAAGCGGAAGGCATGTGGGCGGCAGCTAACGCAGAATTTTCTGAGCCACACGGGTCCTGACCTCATCCC
TGAGGTCCCCCGGCAGCTGGTGACGAACTGCACCCAGCGGCTGGAGCAGGGTCCCTGCAAAGACCTTTTCCAGGAACTCACCCGGCTGA
CCCACGAGTACCTGAGCGTGGCCCCTTTTGCCGACTACCTCGACAGCATCTACTTCAACCGTTTCCTGCAGTGGAAGTGGCTGGAAAGG
CAGCCAGTGACCAAAAACACCTTCAGGCAATACCGAGTCCTGGGCAAAGGTGGCTTTGGGGAGGTGTGCGCCTGCCAGGTGCGGGCCAC
AGGTAAGATGTATGCCTGCAAGAAGCTAGAGAAAAAGCGGATCAAGAAGCGGAAAGGGGAGGCCATGGCGCTGAACGAGAAGCAGATCC
TGGAGAAAGTGAACAGTAGGTTTGTAGTGAGCTTGGCCTACGCCTATGAGACCAAGGACGCGCTGTGCCTGGTGCTGACACTGATGAAC
GGGGGCGACCTCAAGTTCCACATCTACCACATGGGCCAGGCTGGCTTCCCCGAAGCGCGGGCCGTCTTCTACGCCGCCGAGATCTGCTG
TGGCCTGGAGGACCTGCACCGGGAGCGCATCGTGTACAGGGACCTGAAGCCCGAGAACATCTTGCTGGATGACCACGGCCACATCCGCA
TCTCTGACCTGGGACTAGCTGTGCATGTGCCCGAGGGCCAGACCATCAAAGGGCGTGTGGGCACCGTGGGTTACATGGCTCCGGAGGTG
GTGAAGAATGAACGGTACACGTTCAGCCCTGACTGGTGGGCGCTCGGCTGCCTCCTGTACGAGATGATCGCAGGCCAGTCGCCCTTCCA
GCAGAGGAAGAAGAAGATCAAGCGGGAGGAGGTGGAGCGGCTGGTGAAGGAGGTCCCCGAGGAGTATTCCGAGCGCTTTTCCCCGCAGG
CCCGCTCACTTTGCTCACAGCTCCTCTGCAAGGACCCTGCCGAACGCCTGGGGTGTCGTGGGGGCAGTGCCCGCGAGGTGAAGGAGCAC
CCCCTCTTTAAGAAGCTGAACTTCAAGCGGCTGGGAGCTGGCATGCTGGAGCCGCCGTTCAAGCCTGACCCCCAGGCCATTTACTGCAA
GGATGTTCTGGACATTGAACAGTTCTCTACGGTCAAGGGCGTGGAGCTGGAGCCTACCGACCAGGACTTCTACCAGAAGTTTGCCACAG
GCAGTGTGCCCATCCCCTGGCAGAACGAGATGGTGGAGACCGAGTGCTTCCAAGAGCTGAATGTCTTTGGGCTGGATGGCTCAGTTCCC
CCAGACCTGGACTGGAAGGGCCAGCCACCTGCACCTCCTAAAAAGGGACTGCTGCAGAGACTCTTCAGTCGCCAAAGGTGA

SEQ ID NO: 8

Human GRK6 (NM_002082):

MELENIVANTVLLKAREGGGGNRKGKSKKWRQMLQFPHISQCEELRLSLERDYHSLCERHAIGRLLFREFCATRPELSRCVAFLDGVAE
YEVTPDDKRKACGRHVTQNFLSHTGPDLIPEVPRQLVTNCTQRLEQGPCKDLFQELTRLTHEYLSVAPFADYLDSIYFNRFLQWKWLER
QPVTKNTFRQYRVLGKGGFGEVCACQVRATGKMYACKKLEKKRIKKRKGEAMALNEKQILEKVNSRFVVSLAYAYETKDALCLVLTLMN
GGDLKFHIYHMGQAGFPEARAVFYAAEICCGLEDLHRERIVYRDLKPENILLDDHGHIRISDLGLAVHVPEGQTIKGRVGTVGYMAPEV
VKNERYTFSPDWWALGCLLYEMIAGQSPFQQRKKKIKREEVERLVKEVPEEYSERFSPQARSLCSQLLCKDPAERLGCRGGSAREVKEH
PLFKKLNFKRLGAGMLEPPFKPDPQAIYCKDVLDIEQFSTVKGVELEPTDQDFYQKFATGSVPIPWQNEMVETECFQELNVFGLDGSVP
PDLDWKGQPPAPPKKGLLQRLFSRQDCCGNCSDSEEELPTRL

SEQ ID NO: 9

ATGGAGCTCGAGAACATCGTAGCGAACACGGTGCTACTCAAGGCCCGGGAAGGTGGCGGTGGAAATCGCAAAGGCAAAAGCAAGAAATG
GCGGCAGATGCTCCAGTTCCCTCACATCAGCCAGTGCGAAGAGCTGCGGCTCAGCCTCGAGCGTGACTATCACAGCCTGTGCGAGCGGC
ACGCCATTGGGCGCCTGCTGTTCCGAGAGTTCTGTGCCACGAGGCCGGAGCTGAGCCGCTGCGTCGCCTTCCTGGATGGGGTGGCCGAG
TATGAAGTGACCCCGGATGACAAGCGGAAGGCATGTGGGCGGCACGTAACGCAGAATTTTCTGAGCCACACGGGTCCTGACCTCATCCC
TGAGGTCCCCCGGCAGCTGGTGACGAACTGCACCCAGCGGCTGGAGCAGGGTCCCTGCAAAGACCTTTTCCAGGAACTCACCCGGCTGA
CCCACGAGTACCTGAGCGTGGCCCCTTTTGCCGACTACCTCGACAGCATCTACTTCAACCGTTTCCTGCAGTGGAAGTGGCTGGAAAGG
CAGCCAGTGACCAAAAACACCTTCAGGCAATACCGAGTCCTGGGCAAAGGTGGCTTTGGGGAGGTGTGCGCCTGCCAGGTGCGGGCCAC
AGGTAAGATGTATGCCTGCAAGAAGCTAGAGAAAAAGCGGATCAAGAAGCGGAAAGGGGAGGCCATGGCGCTGAACGAGAAGCAGATCC
TGGAGAAAGTGAACAGTAGGTTTGTAGTGAGCTTGGCCTACGCCTATGAGACCAAGGACGCGCTGTGCCTGGTGCTGACACTGATGAAC
GGGGGCGACCTCAAGTTCCACATCTACCACATGGGCCAGGCTGGCTTCCCCGAAGCGCGGGCCGTCTTCTACGCCGCCGAGATCTGCTG
TGGCCTGGAGGACCTGCACCGGGAGCGCATCGTGTACAGGGACCTGAAGCCCGAGAACATCTTGCTGGATGACCACGGCCACATCCGCA
TCTCTGACCTGGGACTAGCTGTGCATGTGCCCGAGGGCCAGACCATCAAAGGGCGTGTGGGCACCGTGGGTTACATGGCTCCGGAGGTG
GTGAAGAATGAACGGTACACGTTCAGCCCTGACTGGTGGGCGCTCGGCTGCCTCCTGTACGAGATGATCGCAGGCCAGTCGCCCTTCCA
GCAGAGGAAGAAGAAGATCAAGCGGGAGGAGGTGGAGCGGCTGGTGAAGGAGGTCCCCGAGGAGTATTCCGAGCGCTTTTCCCCGCAGG
CCCGCTCACTTTGCTCACAGCTCCTCTGCAAGGACCCTGCCGAACGCCTGGGGTGTCGTGGGGGCAGTGCCCGCGAGGTGAAGGAGCAC
CCCCTCTTTAAGAAGCTGAACTTCAAGCGGCTGGGAGCTGGCATGCTGGAGCCGCCGTTCAAGCCTGACCCCCAGGCCATTTACTGCAA
GGATGTTCTGGACATTGAACAGTTCTCTACGGTCAAGGGCGTGGAGCTGGAGCCTACCGACCAGGACTTCTACCAGAAGTTTGCCACAG
GCAGTGTGCCCATCCCCTGGCAGAACGAGATGGTGGAGACCGAGTGCTTCCAAGAGCTGAATGTCTTTGGGCTGGATGGCTCAGTTCCC
CCAGACCTGGACTGGAAGGGCCAGCCACCTGCACCTCCTAAAAAGGGACTGCTGCAGAGACTCTTCAGTCGCCAAGATTGCTGTGGAAA
CTGCAGCGACAGCGAGGAAGAGCTCCCCACCCGCCTCTAGCCCCCAG

SEQ ID NO: 10

Human GRK5 (XM_005969):

MELENIVANTVLLKAREGGGGKRKGKSKKWKEILKFPHISQCEDLRRTIDRDYCSLCDKQPIGRLLFRQFCETRPGLECYIQFLDSVAE
YEVTPDEKLGEKGKEIMTKYLTPKSPVFIAQVGQDLVSQTEEKLLQKPCKELFSACAQSVHEYLRGEPFHEYLDSMFFDRFLQWKWLER
QPVTKNTFRQYRVLGKGGFGEVCACQVRATGKMYACKRLEKKRIKKRKGESMALNEKQILEKVNSQFVVNLAYAYETKDALCLVLTIMN
GGDLKFHIYNMGNPGFEEERALFYAAEILCGLEDLHRENTVYRDLKPENILLDDYGHIRISDLGLAVKIPEGDLIRGRVGTVGYMAPEV
LNNQRYGLSPDYWGLGCLIYEMIEGQSPFRGRKEKVKREEVDRRVLETEEVYSHKFSEEAKSICKMLLTKDAKQRLGCQEEGAAEVKRH
PFFRNMNFKRLEAGMLDPPFVPDPRAVYCKDVLDIEQFSTVKGVNLDHTDDDFYSKFSTGSVSIPWQNEMIETECFKELNVFGPNGTLP
PDLNRNHPPEPPKKGLLQRLFKRQHQNNSKSSPSSKTSFNHHINSNHVSSNSTGSS

SEQ ID NO: 11

ATGGAGCTGGAAAACATCGTGGCCAACACGGTCTTGCTGAAAGCCAGGGAAGGGGGCGGAGGAAAGCGCAAAGGGAAAAGCAAGAAGTG
GAAAGAAATCCTGAAGTTCCCTCACATTAGCCAGTGTGAAGACCTCCGAAGGACCATAGACAGAGATTACTGCAGTTTATGTGACAAGC
AGCCAATCGGGAGGCTGCTTTTCCGGCAGTTTTGTGAAACCAGGCCTGGGCTGGAGTGTTACATTCAGTTCCTGGACTCCGTGGCAGAA
TATGAAGTTACTCCAGATGAAAAACTGGGAGAGAAAGGGAAGGAAATTATGACCAAGTACCTCACCCCAAAGTCCCCTGTTTTCATAGC
CCAAGTTGGCCAAGACCTGGTCTCCCAGACGGAGGAGAAGCTCCTACAGAAGCCGTGCAAAGAACTCTTTTCTGCCTGTGCACAGTCTG
TCCACGAGTACCTGAGGGGAGAACCATTCCACGAATATCTGGACAGCATGTTTTTTGACCGCTTTCTCCAGTGGAAGTGGTTGGAAAGG
CAACCGGTGACCAAAAACACTTTCAGGCAGTATCGAGTGCTAGGAAAAGGGGGCTTCGGGGAGGTCTGTGCCTGCCAGGTTCGGGCCAC
GGGTAAAATGTATGCCTGCAAGCGCTTGGAGAAGAAGAGGATCAAAAAGAGGAAAGGGGAGTCCATGGCCCTCAATGAGAAGCAGATCC
TCGAGAAGGTCAACAGTCAGTTTGTGGTCAACCTGGCCTATGCCTACGAGACCAAGGATGCACTGTGCTTGGTCCTGACCATCATGAAT
GGGGGTGACCTGAAGTTCCACATCTACAACATGGGCAACCCTGGCTTCGAGGAGGAGCGGGCCTTGTTTTATGCGGCAGAGATCCTCTG
CGGCTTAGAAGACCTCCACCGTGAGAACACCGTCTACCGAGATCTGAAACCTGAAAACATCCTGTTAGATGATTATGGCCACATTAGGA
TCTCAGACCTGGGCTTGGCTGTGAAGATCCCCGAGGGAGACCTGATCCGCGGCCGGGTGGGCACTGTTGGCTACATGGCTCCAGAGGTC
CTGAACAACCAGAGGTACGGCCTGAGCCCCGACTACTGGGGCCTTGGCTGCCTCATCTATGAGATGATCGAGGGCCAGTCGCCGTTCCG
CGGCCGCAAGGAGAAGGTGAAGCGGGAGGAGGTGGACCGCCGGGTCCTGGAGACGGAGGAGGTGTACTCCCACAAGTTCTCCGAGGAGG
CCAAGTCCATCTGCAAGATGCTGCTCACGAAAGATGCGAAGCAGAGGCTGGGCTGCCAGGAGGAGGGGGCTGCAGAGGTCAAGAGACAC
CCCTTCTTCAGGAACATGAACTTCAAGCGCTTAGAAGCCGGGATGTTGGACCCTCCCTTCGTTCCAGACCCCCGCGCTGTGTACTGTAA
GGACGTGCTGGACATCGAGCAGTTCTCCACTGTGAAGGGCGTCAATCTGGACCACACAGACGACGACTTCTACTCCAAGTTCTCCACGG
GCTCTGTGTCCATCCCATGGCAAAACGAGATGATAGAAACAGAATGCTTTAAGGAGCTGAACGTGTTTGGACCTAATGGTACCCTCCCG
CCAGATCTGAACAGAAACCACCCTCCGGAACCGCCCAAGAAAGGGCTGCTCCAGAGACTCTTCAAGCGGCAGCATCAGAACAATTCCAA
GAGTTCGCCCAGCTCCAAGACCAGTTTTAACCACCACATAAACTCAAACCATGTCAGCTCGAACTCCACCGGAAGCAGCTAG

SEQ ID NO: 12

Human GRK4 δ Splice Variant (NM_005307, L03718), also named GRK4B:

MELENIVANSLLLKARQEKDYSSLCDKQPIGRRLFRQFCDTKPTLKRHIEFLDAVAEYEVADDEDRSDCGLSILDRFFNDKLAAPLPEI
PPDVVTECRLGLKEENPSKKAFEECTRVAHNYLRGEPFEEYQESSYFSQFLQWKWLERQPVTKNTFRHYRVLGKGGFGEVCACQVRATG
KMYACKKLQKKRIKKRKGEAMALNEKRILEKVQSRFVVSLAYAYETKDALCLVLTIMNGGDLKFHIYNLGNPGFDEQRAVFYAAELCCG
LEDLQRERIVYRDLKPENILLDDRGHIRISDLGLATEIPEGQRVRGRVGTVGYMAPEVVNNEKYTFSPDWWGLGCLIYEMIQGHSPFKK
YKEKVKWEEVDQRIKNDTEEYSEKFSEDAKSICRMLLTKNPSKRLGCRGEGAAGVKQHPVFKDINFRRLEANMLEPPFCPDPHAVYCKD
VLDIEQFSAVKGIYLDTADEDFYARFATGCVSIPWQNEDCLTMVPSEKEVEPKQC

SEQ ID NO: 13

ATGGAGCTCGAGAACATCGTGGCCAACTCGCTGCTGCTGAAAGCGCGTCAAGAAAAGGATTATAGCAGTCTTTGTGACAAGCAACCGAT
AGGAAGACGTCTCTTCAGGCAGTTCTGTGATACCAAACCCACTCTAAAGAGGCACATTGAATTCTTGGATGCAGTGGCAGAATATGAAG
TTGCCGATGATGAGGACCGAAGTGATTGTGGACTGTCAATCTTAGATAGATTCTTCAATGATAAGTTGGCAGCCCCTTTACCAGAAATA
CCTCCAGATGTTGTGACAGAATGTAGATTGGGACTGAAGGAGGAGAACCCTTCCAAAAAAGCCTTTGAGGAATGTACTAGAGTTGCCCA
TAACTACCTAAGAGGGGAACCATTTGAAGAATACCAAGAAAGCTCATATTTTTCTCAGTTTTTACAATGGAAATGGCTGGAAAGGCAAC
CCGTAACAAAGAACACATTTAGACATTACAGAGTTCTAGGAAAAGGCGGATTTGGAGAGGTTTGCGCCTGTCAAGTGCGAGCCACAGGA
AAAATGTATGCCTGCAAAAAGCTACAAAAAAAAAGAATAAAGAAGAGGAAAGGTGAAGCTATGGCTCTAAATGAGAAAAGAATTCTGGA
GAAAGTGCAAAGTAGATTCGTAGTTAGTTTAGCCTACGCTTATGAAACCAAAGATGCCTTGTGCTTGGTGCTCACCATTATGAATGGAG
GGGATTTGAAGTTTCACATTTACAACCTGGGCAATCCCGGCTTTGATGAGCAGAGAGCCGTTTTCTATGCTGCAGAGCTGTGTTGCGGC
TTGGAAGATTTACAGAGGGAAAGAATTGTATACAGAGACTTGAAGCCTGAGAATATTCTCCTTGATGATCGTGGACACATCCGGATTTC
AGACCTCGGTTTGGCCACAGAGATCCCAGAAGGACAGAGGGTTCGAGGAAGAGTTGGAACAGTCGGCTACATGGCACCTGAAGTTGTCA
ATAATGAAAAGTATACGTTTAGTCCCGATTGGTGGGGACTTGGCTGTCTGATCTATGAAATGATTCAGGGACATTCTCCATTCAAAAAA
TACAAAGAGAAAGTCAAATGGGAGGAGGTCGATCAAAGAATCAAGAATGATACCGAGGAGTATTCTGAGAAGTTTTCAGAGGATGCCAA
ATCTATCTGCAGGATGTTACTCACCAAGAATCCAAGCAAGCGGCTGGGCTGCAGGGGCGAGGGAGCGGCTGGGGTGAAGCAGCACCCCG
TGTTCAAGGACATCAACTTCAGGAGGCTGGAGGCAAACATGCTGGAGCCCCCTTTCTGTCCTGATCCTCATGCCGTTTACTGTAAGGAC
GTCCTGGATATCGAGCAGTTCTCGGCGGTGAAAGGGATCTACCTGGACACCGCAGATGAAGACTTCTATGCTCGGTTTGCTACCGGGTG
TGTCTCCATCCCCTGGCAGAATGAGGACTGCCTGACCATGGTCCCCAGTGAGAAGGAAGTGGAACCCAAGCAATGCTGA

SEQ ID NO: 14

Human GRK4 β Splice Variant (U33055, X97880), also named GRK4C:

MELENIVANSLLLKARQEKDYSSLCDKQPIGRRLFRQFCDTKPTLKRHIEFLDAVAEYEVADDEDRSDCGLSILDRFFNDKLAAPLPEI
PPDVVTECRLGLKEENPSKKAFEECTRVAHNYLRGEPFEEYQESSYFSQFLQWKWLERQPVTKNTFRHYRVLGKGGFGEVCACQVRATG
KMYACKKLQKKRIKKRKGEAMALNEKRILEKVQSRFVVSLAYAYETKDALCLVLTIMNGGDLKFHIYNLGNPGFDEQRAVFYAAELCCG
LEDLQRERIVYRDLKPENILLDDRGHIRISDLGLATEIPEGQRVRGRVGTVGYMAPEVVNNEKYTFSPDWWGLGCLIYEMIQGHSPFKK
YKEKVKWEEVDQRIKNDTEEYSEKFSEDAKSICRMLLTKNPSKRLGCRGEGAAGVKQHPVFKDINFRRLEANMLEPPFCPDPHAVYCKD
VLDIEQFSAVKGIYLDTADEDFYARFATGCVSIPWQNEMIESGCFKDINKSESEEALPLDLDKNIHTPVSRPNRGFFYRLFRRGGCLTM
VPSEKEVEPKQC

SEQ ID NO: 15

ATGGAGCTCGAGAACATCGTGGCCAACTCGCTGCTGCTGAAAGCGCGTCAAGAAAAGGATTATAGCAGTCTTTGTGACAAGCAACCGAT
AGGAAGACGTCTCTTCAGGCAGTTCTGTGATACCAAACCCACTCTAAAGAGGCACATTGAATTCTTGGATGCAGTGGCAGAATATGAAG
TTGCCGATGATGAGGACCGAAGTGATTGTGGACTGTCAATCTTAGATAGATTCTTCAATGATAAGTTGGCAGCCCCTTTACCAGAAATA
CCTCCAGATGTTGTGACAGAATGTAGATTGGGACTGAAGGAGGAGAACCCTTCCAAAAAAGCCTTTGAGGAATGTACTAGAGTTGCCCA
TAACTACCTAAGAGGGGAACCATTTGAAGAATACCAAGAAAGCTCATATTTTTCTCAGTTTTTACAATGGAAATGGCTGGAAAGGCAAC
CCGTAACAAAGAACACATTTAGACATTACAGAGTTCTAGGAAAAGGCGGATTTGGAGAGGTTTGCGCCTGTCAAGTGCGAGCCACAGGA
AAAATGTATGCCTGCAAAAAGCTACAAAAAAAAAGAATAAAGAAGAGGAAAGGTGAAGCTATGGCTCTAAATGAGAAAAGAATTCTGGA
GAAAGTGCAAAGTAGATTCGTAGTTAGTTTAGCCTACGCTTATGAAACCAAAGATGCCTTGTGCTTGGTGCTCACCATTATGAATGGAG
GGGATTTGAAGTTTCACATTTACAACCTGGGCAATCCCGGCTTTGATGAGCAGAGAGCCGTTTTCTATGCTGCAGAGCTGTGTTGCGGC
TTGGAAGATTTACAGAGGGAAAGAATTGTATACAGAGACTTGAAGCCTGAGAATATTCTCCTTGATGATCGTGGACACATCCGGATTTC
AGACCTCGGTTTGGCCACAGAGATCCCAGAAGGACAGAGGGTTCGAGGAAGAGTTGGAACAGTCGGCTACATGGCACCTGAAGTTGTCA
ATAATGAAAAGTATACGTTTAGTCCCGATTGGTGGGGACTTGGCTGTCTGATCTATGAAATGATTCAGGGACATTCTCCATTCAAAAAA
TACAAAGAGAAAGTCAAATGGGAGGAGGTCGATCAAAGAATCAAGAATGATACCGAGGAGTATTCTGAGAAGTTTTCAGAGGATGCCAA
ATCTATCTGCAGGATGTTACTCACCAAGAATCCAAGCAAGCGGCTGGGCTGCAGGGGCGAGGGAGCGGCTGGGGTGAAGCAGCACCCCG
TGTTCAAGGACATCAACTTCAGGAGGCTGGAGGCAAACATGCTGGAGCCCCCTTTCTGTCCTGATCCTCATGCCGTTTACTGTAAGGAC
GTCCTGGATATCGAGCAGTTCTCGGCGGTGAAAGGGATCTACCTGGACACCGCAGATGAAGACTTCTATGCTCGGTTTGCTACCGGGTG
TGTCTCCATCCCCTGGCAGAATGAGATGATCGAATCCGGGTGTTTCAAAGACATCAACAAAAGTGAAAGTGAGGAAGCTTTGCCATTAG
ATCTAGACAAGAACATACATACCCCGGTTTCCAGACCAAACAGAGGCTTCTTCTATAGACTCTTCAGAAGAGGGGGCTGCCTGACCATG
GTCCCCAGTGAGAAGGAAGTGGAACCCAAGCAATGCTGA

SEQ ID NO: 16

Human GRK4 α Splice Variant (U33054, X97881), also named GRK4D:

MELENIVANSLLLKARQGGYGKKSGRSKKWKEILTLPPVSQCSELRHSIEKDYSSLCDKQPIGRRLFRQFCDTKPTLKRHIEFLDAVAE
YEVADDEDRSDCGLSILDRFFNDKLAAPLPEIPPDVVTECRLGLKEENPSKKAFEECTRVAHNYLRGEPFEEYQESSYFSQFLQWKWLE
RQPVTKNTFRHYRVLGKGGFGEVCACQVRATGKMYACKKLQKKRIKKRKGEAMALNEKRILEKVQSRFVVSLAYAYETKDALCLVLTIM
NGGDLKFHIYNLGNPGFDEQRAVFYAAELCCGLEDLQRERIVYRDLKPENILLDDRGHIRISDLGLATEIPEGQRVRGRVGTVGYMAPE
VVNNEKYTFSPDWWGLGCLIYEMIQGHSPFKKYKEKVKWEEVDQRIKNDTEEYSEKFSEDAKSICRMLLTKNPSKRLGCRGEGAAGVKQ
HPVFKDINFRRLEANMLEPPFCPDPHAVYCKDVLDIEQFSAVKGIYLDTADEDFYARFATGCVSIPWQNEMIESGCFKDINKSESEEAL
PLDLDKNIHTPVSRPNRGFFYRLFRRGGCLTMVPSEKEVEPKQC

SEQ ID NO: 17

ATGGAGCTCGAGAACATCGTGGCCAACTCGCTGCTGCTGAAAGCGCGTCAAGGAGGATATGGCAAAAAAAGTGGTCGTAGTAAAAAATG
GAAGGAGATACTGACACTGCCTCCTGTCAGCCAGTGCAGTGAGCTTAGACATTCCATTGAAAAGGATTATAGCAGTCTTTGTGACAAGC
AACCGATAGGAAGACGTCTCTTCAGGCAGTTCTGTGATACCAAACCCACTCTAAAGAGGCACATTGAATTCTTGGATGCAGTGGCAGAA
TATGAAGTTGCCGATGATGAGGACCGAAGTGATTGTGGACTGTCAATCTTAGATAGATTCTTCAATGATAAGTTGGCAGCCCCTTTACC
AGAAATACCTCCAGATGTTGTGACAGAATGTAGATTGGGACTGAAGGAGGAGAACCCTTCCAAAAAAGCCTTTGAGGAATGTACTAGAG
TTGCCCATAACTACCTAAGAGGGGAACCATTTGAAGAATACCAAGAAAGCTCATATTTTTCTCAGTTTTTACAATGGAAATGGCTGGAA
AGGCAACCCGTAACAAAGAACACATTTAGACATTACAGAGTTCTAGGAAAAGGCGGATTTGGAGAGGTTTGCGCCTGTCAAGTGCGAGC
CACAGGAAAAATGTATGCCTGCAAAAAGCTACAAAAAAAAAGAATAAAGAAGAGGAAAGGTGAAGCTATGGCTCTAAATGAGAAAAGAA
TTCTGGAGAAAGTGCAAAGTAGATTCGTAGTTAGTTTAGCCTACGCTTATGAAACCAAAGATGCCTTGTGCTTGGTGCTCACCATTATG
AATGGAGGGGATTTGAAGTTTCACATTTACAACCTGGGCAATCCCGGCTTTGATGAGCAGAGAGCCGTTTTCTATGCTGCAGAGCTGTG
TTGCGGCTTGGAAGATTTACAGAGGGAAAGAATTGTATACAGAGACTTGAAGCCTGAGAATATTCTCCTTGATGATCGTGGACACATCC
GGATTTCAGACCTCGGTTTGGCCACAGAGATCCCAGAAGGACAGAGGGTTCGAGGAAGAGTTGGAACAGTCGGCTACATGGCACCTGAA
GTTGTCAATAATGAAAAGTATACGTTTAGTCCCGATTGGTGGGGACTTGGCTGTCTGATCTATGAAATGATTCAGGGACATTCTCCATT
CAAAAAATACAAAGAGAAAGTCAAATGGGAGGAGGTCGATCAAAGAATCAAGAATGATACCGAGGAGTATTCTGAGAAGTTTTCAGAGG
ATGCCAAATCTATCTGCAGGATGTTACTCACCAAGAATCCAAGCAAGCGGCTGGGCTGCAGGGGCGAGGGAGCGGCTGGGGTGAAGCAG
CACCCCGTGTTCAAGGACATCAACTTCAGGAGGCTGGAGGCAAACATGCTGGAGCCCCCTTTCTGTCCTGATCCTCATGCCGTTTACTG
TAAGGACGTCCTGGATATCGAGCAGTTCTCGGCGGTGAAAGGGATCTACCTGGACACCGCAGATGAAGACTTCTATGCTCGGTTTGCTA
CCGGGTGTGTCTCCATCCCCTGGCAGAATGAGATGATCGAATCCGGGTGTTTCAAAGACATCAACAAAAGTGAAAGTGAGGAAGCTTTG
CCATTAGATCTAGACAAGAACATACATACCCCGGTTTCCAGACCAAACAGAGGCTTCTTCTATAGACTCTTCAGAAGAGGGGGCTGCCT
GACCATGGTCCCCAGTGAGAAGGAAGTGGAACCCAAGCAATGCTGA

SEQ ID NO: 18

Human GRK4 γ Splice Variant (U33056):

MELENIVANSLLLKARQGGYGKKSGRSKKWKEILTLPPVSQCSELRHSIEKDYSSLCDKQPIGRRLFRQFCDTKPTLKRHIEFLDAVAE
YEVADDEDRSDCGLSILDRFFNDKLAAPLPEIPPDVVTECRLGLKEENPSKKAFEECTRVAHNYLRGEPFEEYQESSYFSQFLQWKWLE
RQPVTKNTFRHYRVLGKGGFGEVCACQVRATGKMYACKKLQKKRIKKRKGEAMALNEKRILEKVQSRFVVSLAYAYETKDALCLVLTIM
NGGDLKFHIYNLGNPGFDEQRAVFYAAELCCGLEDLQRERIVYRDLKPENILLDDRGHIRISDLGLATEIPEGQRVRGRVGTVGYMAPE
VVNNEKYTFSPDWWGLGCLIYEMIQGHSPFKKYKEKVKWEEVDQRIKNDTEEYSEKFSEDAKSICRMLLTKNPSKRLGCRGEGAAGVKQ
HPVFKDINFRRLEANMLEPPFCPDPHAVYCKDVLDIEQFSAVKGIYLDTADEDFYARFATGCVSIPWQNEGCLTMVPSEKEVEPKQC

SEQ ID NO: 19

ATGGAGCTCGAGAACATCGTGGCCAACTCGCTGCTGCTGAAAGCGCGTCAAGGAGGATATGGCAAAAAAAGTGGTCGTAGTAAAAAATG
GAAGGAGATACTGACACTGCCTCCTGTCAGCCAGTGCAGTGAGCTTAGACATTCCATTGAAAAGGATTATAGCAGTCTTTGTGACAAGC
AACCGATAGGAAGACGTCTCTTCAGGCAGTTCTGTGATACCAAACCCACTCTAAAGAGGCACATTGAATTCTTGGATGCAGTGGCAGAA
TATGAAGTTGCCGATGATGAGGACCGAAGTGATTGTGGACTGTCAATCTTAGATAGATTCTTCAATGATAAGTTGGCAGCCCCTTTACC
AGAAATACCTCCAGATGTTGTGACAGAATGTAGATTGGGACTGAAGGAGGAGAACCCTTCCAAAAAAGCCTTTGAGGAATGTACTAGAG
TTGCCCATAACTACCTAAGAGGGGAACCATTTGAAGAATACCAAGAAAGCTCATATTTTTCTCAGTTTTTACAATGGAAATGGCTGGAA
AGGCAACCCGTAACAAAGAACACATTTAGACATTACAGAGTTCTAGGAAAAGGCGGATTTGGAGAGGTTTGCGCCTGTCAAGTGCGAGC
CACAGGAAAAATGTATGCCTGCAAAAAGCTACAAAAAAAAAGAATAAAGAAGAGGAAAGGTGAAGCTATGGCTCTAAATGAGAAAAGAA
TTCTGGAGAAAGTGCAAAGTAGATTCGTAGTTAGTTTAGCCTACGCTTATGAAACCAAAGATGCCTTGTGCTTGGTGCTCACCATTATG
AATGGAGGGGATTTGAAGTTTCACATTTACAACCTGGGCAATCCCGGCTTTGATGAGCAGAGAGCCGTTTTCTATGCTGCAGAGCTGTG
TTGCGGCTTGGAAGATTTACAGAGGGAAAGAATTGTATACAGAGACTTGAAGCCTGAGAATATTCTCCTTGATGATCGTGGACACATCC
GGATTTCAGACCTCGGTTTGGCCACAGAGATCCCAGAAGGACAGAGGGTTCGAGGAAGAGTTGGAACAGTCGGCTACATGGCACCTGAA
GTTGTCAATAATGAAAAGTATACGTTTAGTCCCGATTGGTGGGGACTTGGCTGTCTGATCTATGAAATGATTCAGGGACATTCTCCATT
CAAAAAATACAAAGAGAAAGTCAAATGGGAGGAGGTCGATCAAAGAATCAAGAATGATACCGAGGAGTATTCTGAGAAGTTTTCAGAGG
ATGCCAAATCTATCTGCAGGATGTTACTCACCAAGAATCCAAGCAAGCGGCTGGGCTGCAGGGGCGAGGGAGCGGCTGGGGTGAAGCAG
CACCCCGTGTTCAAGGACATCAACTTCAGGAGGCTGGAGGCAAACATGCTGGAGCCCCCTTTCTGTCCTGATCCTCATGCCGTTTACTG
TAAGGACGTCCTGGATATCGAGCAGTTCTCGGCGGTGAAAGGGATCTACCTGGACACCGCAGATGAAGACTTCTATGCTCGGTTTGCTA
CCGGGTGTGTCTCCATCCCCTGGCAGAATGAGGGCTGCCTGACCATGGTCCCCAGTGAGAAGGAAGTGGAACCCAAGCAATGCTGA

SEQ ID NO: 20

Human GRK 7 (NM_139209):

MVDMGALDNLIANTAYLQARKPSDCDSKELQRRRRSLALPGLQGCAELRQKLSLNFHSLCEQQPIGRRLFRDFLATVPTFRKAATFLED
VQNWELAEEGPTKDSALQGLVATCASAPAPGNPQPFLSQAVATKCQAATTEEERVAAVTLAKAEAMAFLQEQPFKDFVTSAFYDKFLQW
KLFEMQPVSDKYFTEFRVLGKGGFGEVCAVQVKNTGKMYACKKLDKKRLKKKGGEKMALLEKEILEKVSSPFIVSLAYAFESKTHLCLV
MSLMNGGDLKFHIYNVGTRGLDMSRVIFYSAQIACGMLHLHELGIVYRDMKPENVLLDDLGNCRLSDLGLAVEMKGGKPITQRAGTNGY
MAPEILMEKVSYSYPVDWFAMGCSIYEMVAGRTPFKDYKEKVSKEDLKQRTLQDEVKFQHDNFTEEAKDICRLFLAKKPEQRLGSREKS
DDPRKHHFFKTINFPRLEAGLIEPPFVPDPSVVYAKDIAEIDDFSEVRGVEFDDKDKQFFKNFATGAVPIAWQEEIIETGLFEELNDPN
RPTGCEEGNSSKSGVCLLL

SEQ ID NO: 21

ATGGTGGACATGGGGGCCCTGGACAACCTGATCGCCAACACCGCCTACCTGCAGGCCCGGAAGCCCTCGGACTGCGACAGCAAAGAGCT
GCAGCGGCGGCGGCGTAGCCTGGCCCTGCCCGGGCTGCAGGGCTGCGCGGAGCTCCGCCAGAAGCTGTCCCTGAACTTCCACAGCCTGT
GTGAGCAGCAGCCCATCGGTCGCCGCCTCTTCCGTGACTTCCTAGCCACAGTGCCCACGTTCCGCAAGGCGGCAACCTTCCTAGAGGAC
GTGCAGAACTGGGAGCTGGCCGAGGAGGGACCCACCAAAGACAGCGCGCTGCAGGGGCTGGTGGCCACTTGTGCGAGTGCCCCTGCCCC
GGGGAACCCGCAACCCTTCCTCAGCCAGGCCGTGGCCACCAAGTGCCAAGCAGCCACCACTGAGGAAGAGCGAGTGGCTGCAGTGACGC
TGGCCAAGGCTGAGGCCATGGCTTTCTTGCAAGAGCAGCCCTTTAAGGATTTCGTGACCAGCGCCTTCTACGACAAGTTTCTGCAGTGG
AAACTCTTCGAGATGCAACCAGTGTCAGACAAGTACTTCACTGAGTTCAGAGTGCTGGGGAAAGGTGGTTTTGGGGAGGTATGTGCCGT
CCAGGTGAAAAACACTGGGAAGATGTATGCCTGTAAGAAACTGGACAAGAAGCGGCTGAAGAAGAAAGGTGGCGAGAAGATGGCTCTCT
TGGAAAAGGAAATCTTGGAGAAGGTCAGCAGCCCTTTCATTGTCTCTCTGGCCTATGCCTTTGAGAGCAAGACCCATCTCTGCCTTGTC
ATGAGCCTGATGAATGGGGGAGACCTCAAGTTCCACATCTACAACGTGGGCACGCGTGGCCTGGACATGAGCCGGGTGATCTTTTACTC
GGCCCAGATAGCCTGTGGGATGCTGCACCTCCATGAACTCGGCATCGTCTATCGGGACATGAAGCCTGAGAATGTGCTTCTGGATGACC
TCGGCAACTGCAGGTTATCTGACCTGGGGCTGGCCGTGGAGATGAAGGGTGGCAAGCCCATCACCCAGAGGGCTGGAACCAATGGTTAC
ATGGCTCCTGAGATCCTAATGGAAAAGGTAAGTTATTCCTATCCTGTGGACTGGTTTGCCATGGGATGCAGCATTTATGAAATGGTTGC
TGGACGAACACCATTCAAAGATTACAAGGAAAAGGTCAGTAAAGAGGATCTGAAGCAAAGAACTCTGCAAGACGAGGTCAAATTCCAGC
ATGATAACTTCACAGAGGAAGCAAAAGATATTTGCAGGCTCTTCTTGGCTAAGAAACCAGAGCAACGCTTAGGAAGCAGAGAAAAGTCT
GATGATCCCAGGAAACATCATTTCTTTAAAACGATCAACTTTCCTCGCCTGGAAGCTGGCCTAATTGAACCCCCATTTGTGCCAGACCC
TTCAGTGGTTTATGCCAAAGACATCGCTGAAATTGATGATTTCTCTGAGGTTCGGGGGGTGGAATTTGATGACAAAGATAAGCAGTTCT
TCAAAAACTTTGCGACAGGTGCTGTTCCTATAGCATGGCAGGAAGAAATTATAGAAACGGGACTGTTTGAGGAACTGAATGACCCCAAC
AGACCTACGGGTTGTGAGGAGGGTAATTCATCCAAGTCTGGCGTGTGTTTGTTATTGTAA

SEQ ID NO: 22

Human Rhodopsin Kinase (GRK1) (NM_002929):

MDFGSLETVVANSAFIAARGSFDGSSSQPSRDKKYLAKLKLPPLSKCESLRDSLSLEFESVCLEQPIGKKLFQQFLQSAEKHLPALELW
KDIEDYDTADNDLQPQKAQTILAQYLDPQAKLFCSFLDEGIVAKFKEGPVEIQDGLFQPLLQATLAHLGQAPFQEYLGSLYFLRFLQWK
WLEAQPMGEDWFLDFRVLGKGGFGEVSACQMKATGKLYACKKLNKKRLKKRKGYQGAMVEKKILMKVHSRFIVSLAYAFETKADLCLVM
TIMNGGDIRYHIYNVNEENPGFPEPRALFYTAQIICGLEHLHQRRIVYRDLKPENVLLDNDGNVRISDLGLAVELLDGQSKTKGYAGTP
GFMAPELLQGEEYDFSVDYFALGVTLYEMIAARGPFRARGEKVENKELKHRIISEPVKYPDKFSQASKDFCEALLEKDPEKRLGFRDET
CDKLRAHPLFKDLNWRQLEAGMLMPPFIPDSKTVYAKDIQDVGAFSTVKGVAFDKTDTEFFQEFATGNCPIPWQEEMIETGIFGELNVW
RSDGQMPDDMKGISGGSSSSSKSGMCLVS

SEQ ID NO: 23

ATGGATTTCGGGTCTTTGGAGACCGTGGTGGCCAACTCTGCCTTCATCGCCGCCCGAGGCAGCTTTGACGGCAGCAGCTCCCAACCCTC
CCGGGACAAGAAGTACCTGGCCAAGCTCAAGCTGCCCCCGCTGTCCAAGTGTGAGTCCCTCCGCGACAGCCTCAGCCTGGAGTTTGAGA
GTGTGTGCTTGGAGCAGCCCATCGGCAAGAAGCTCTTTCAGCAGTTCCTACAATCGGCAGAGAAGCACCTGCCGGCCCTGGAGCTCTGG
AAAGACATCGAGGACTATGACACGGCAGACAATGACCTCCAGCCACAGAAGGCCCAGACCATCCTGGCCCAGTACCTGGACCCCCAGGC
CAAACTCTTCTGCAGCTTCCTGGATGAGGGGATAGTGGCGAAGTTTAAGGAGGGGCCTGTGGAGATCCAGGACGGGCTCTTCCAGCCCC
TGCTGCAGGCCACCCTGGCACACCTGGGCCAAGCCCCCTTCCAGGAGTACCTGGGCAGCCTGTACTTCCTGAGGTTCCTGCAGTGGAAG
TGGCTGGAAGCCCAGCCCATGGGGGAGGACTGGTTCCTGGACTTCAGGGTCCTGGGGAAAGGGGGCTTCGGGGAGGTGTCGGCCTGCCA
GATGAAGGCGACCGGCAAGCTGTATGCCTGCAAGAAGCTGAACAAGAAGCGGCTGAAGAAGAGGAAGGGCTACCAGGGTGCTATGGTGG
AGAAGAAGATTCTGATGAAAGTACACAGCAGGTTCATCGTGTCTCTGGCCTATGCGTTTGAAACCAAAGCCGACCTCTGTCTGGTGATG
ACCATCATGAACGGAGGTGACATCAGGTACCACATCTACAACGTGAATGAGGAGAACCCTGGCTTCCCGGAGCCGCGCGCCCTCTTCTA
CACGGCGCAGATCATCTGCGGCCTGGAGCACCTGCACCAGAGGCGGATCGTCTACCGCGACCTCAAGCCCGAGAACGTGCTGCTGGACA
ATGACGGCAATGTCCGGATCTCTGACCTTGGGCTGGCCGTGGAGCTGCTGGACGGACAGAGCAAGACCAAGGGCTACGCAGGGACCCCA
GGTTTCATGGCCCCCGAGCTCCTGCAGGGCGAGGAGTACGACTTCTCCGTGGACTACTTTGCCCTGGGGGTCACCCTGTATGAGATGAT
TGCGGCCAGAGGACCCTTCCGAGCCCGTGGAGAGAAGGTGGAGAACAAGGAGCTGAAGCACCGGATCATCTCAGAGCCCGTGAAGTACC
CTGATAAGTTCAGCCAGGCCAGCAAGGACTTCTGCGAGGCGCTGCTGGAGAAGGACCCGGAGAAGCGCCTGGGGTTCAGAGATGAGACC
TGCGACAAGCTCCGTGCCCACCCCCTCTTCAAGGACCTTAACTGGAGGCAGCTGGAGGCTGGGATGCTGATGCCCCCTTTCATCCCAGA
CTCCAAAACTGTCTACGCAAAGGATATTCAGGACGTGGGTGCCTTTTCCACCGTCAAAGGTGTGGCCTTTGACAAAACAGACACAGAAT
TCTTTCAGGAATTTGCCACTGGCAACTGCCCCATCCCCTGGCAGGAGGAGATGATCGAGACGGGCATCTTTGGCGAGCTGAACGTGTGG
CGCTCGGACGGTCAGATGCCGGACGACATGAAGGGCATCTCCGGGGGCTCCAGCTCCTCGTCCAAGTCAGGGATGTGTCTGGTTTCCTA
G

SEQ ID NO: 24

Human GRK3 (β -aderengic receptor kinase 2) (XM_037826):

MADLEAVLADVSYLMAMEKSKATPAARASKRIVLPEPSIRSVMQKYLAERNEITFDKIFNQKIGFLLFKDFCLNEINEAVPQVKFYEEIKEYEK
LDNEEDRLCRSRQIYDAYIMKELLSCSHPFSKQAVEHVQSHLSKKQVTSTLFQPYIEEICESLRGDIFQKFMESDKFTRFCQWKNVELNIHLTM
NEFSVHRIIGRGGFGEVYGCRKADTGKMYAMKCLDKKRIKMKQGETLALNERIMLSLVSTGDCPFIVCMTYAFHTPDKLCFILDLMNGGDLHYH
LSQHGVFSEKEMRFYATEIILGLEHMHNRFVVYRDLKPANILLDEHGHARISDLGLACDFSKKKPHASVGTHGYMAPEVLQKGTAYDSSADWFS
LGCMLFKLLRGHSPFRQHKTKDKHEIDRMTLTVNVELPDTFSPELKSLLEGLLQRDVSKRLGCHGGGSQEVKEHSFFKGVDWQHVYLQKYPPPL
IPPRGEVNAADAFDIGSFDEEDTKGIKLLDCDQELYKNFPLVISERWQQEVTETVYEAVNADTDKIEARKRAKNKQLGHEEDYALGKDCIMHGY
MLKLGNPFLTQWQRRYFYLFPNRLEWRGEGESRQNLLTMEQILSVEETQIKDKKCILFRIKGGKQFVLQCESDPEFVQWKKELNETFKEAQRLL
RRAPKFLNKPRSGTVELPKPSLCHRNSNGL

SEQ ID NO: 25

ATGGCGGACCTGGAGGCTGTGCTGGCCGATGTCAGTTACCTGATGGCCATGGAGAAGAGCAAGGCGACCCCGGCCGCCCGCGCCAGCAAGAGGA
TCGTCCTGCCGGAGCCCAGTATCCGGAGTGTGATGCAGAAGTACCTTGCAGAGAGAAATGAAATAACCTTTGACAAGATTTTCAATCAGAAAAT
TGGTTTCTTGCTATTTAAAGATTTTTGTTTGAATGAAATTAATGAAGCTGTACCTCAGGTGAAGTTTTATGAAGAGATAAAGGAATATGAAAAA
CTTGATAATGAGGAAGACCGCCTTTGCAGAAGTCGACAAATTTATGATGCCTACATCATGAAGGAACTTCTTTCCTGTTCACATCCTTTCTCAA
AGCAAGCTGTAGAACACGTACAAAGTCATTTATCCAAGAAACAAGTGACATCAACTCTTTTTCAGCCATACATAGAAGAAATTTGTGAAAGCCT
TCGAGGTGACATTTTTCAAAAATTTATGGAAAGTGACAAGTTCACTAGATTTTGTCAGTGGAAAAACGTTGAATTAAATATCCATTTGACCATG
AATGAGTTCAGTGTGCATAGGATTATTGGACGAGGAGGATTCGGGGAAGTTTATGGTTGCAGGAAAGCAGACACTGGAAAAATGTATGCAATGA
AATGCTTAGATAAGAAGAGGATCAAAATGAAACAAGGAGAAACATTAGCCTTAAATGAAAGAATCATGTTGTCTCTTGTCAGCACAGGAGACTG
TCCTTTCATTGTATGTATGACCTATGCCTTCCATACCCCAGATAAACTCTGCTTCATCCTGGATCTGATGAACGGGGGCGATTTGCACTACCAC
CTTTCACAACACGGTGTGTTCTCTGAGAAGGAGATGCGGTTTTATGCCACTGAAATCATTCTGGGTCTGGAACACATGCACAATCGGTTTGTTG
TCTACAGAGATTTGAAGCCAGCAAATATTCTCTTGGATGAACATGGACACGCAAGAATATCAGATCTTGGTCTTGCCTGCGATTTTTCCAAAAA
GAAGCCTCATGCGAGTGTTGGCACCCATGGGTACATGGCTCCCGAGGTGCTGCAGAAGGGGACGGCCTATGACAGCAGTGCCGACTGGTTCTCC
CTGGGCTGCATGCTTTTCAAACTTCTGAGAGGTCACAGCCCTTTCAGACAACATAAAACCAAAGACAAGCATGAAATTGACCGAATGACACTCA
CCGTGAATGTGGAACTTCCAGACACCTTCTCTCCTGAACTGAAGTCCCTTTTGGAGGGCTTGCTTCAGCGAGACGTTAGCAAGCGGCTGGGCTG
TCACGGAGGCGGCTCACAGGAAGTAAAAGAGCACAGCTTTTTCAAAGGTGTTGACTGGCAGCATGTCTACTTACAAAAGTACCCACCACCCTTG
ATTCCTCCCCGGGGAGAAGTCAATGCTGCTGATGCCTTTGATATTGGCTCATTTGATGAAGAGGATACCAAAGGGATTAAGCTACTTGATTGCG
ACCAAGAACTCTACAAGAACTTCCCTTTGGTCATCTCTGAACGCTGGCAGCAAGAAGTAACGGAAACAGTTTATGAAGCAGTAAATGCAGACAC
AGATAAAATCGAGGCCAGGAAGAGAGCTAAAAATAAGCAACTTGGCCACGAAGAAGATTACGCTCTGGGGAAGGACTGTATTATGCACGGGTAC
ATGCTGAAACTGGGAAACCCATTTCTGACTCAGTGGCAGCGTCGCTATTTTTACCTCTTTCCAAATAGACTTGAATGGAGAGGAGAGGGAGAGT
CCCGGCAAAATTTACTGACAATGGAACAGATTCTCTCTGTGGAAGAAACTCAAATTAAAGACAAAAAATGCATTTTGTTCAGAATAAAAGGAGG
GAAACAATTTGTCTTGCAATGTGAGAGTGATCCAGAGTTTGTGCAGTGGAAGAAAGAGTTGAACGAAACCTTCAAGGAGGCCCAGCGGCTATTG
CGTCGTGCCCCGAAGTTCCTCAACAAACCTCGGTCAGGTACTGTGGAGCTCCCAAAGCCATCCCTCTGTCACAGAAACAGCAACGGCCTCTAG

SEQ ID NO: 26

Human GRK1b Splice Variants (AF019764 and AF019765)

Bovine GRK3 (β-adenergic receptor kinase 2) (M73216):

MADLEAVLADVSYLMAMEKSKATPAARASKKIVLPEPSIRSVMQKYLEERHEITFDKIFNQRIGFLLFKDFCLNEINEAVPQVKFYEEI
KEYEKLENEEDRLCRSRQIYDTYIMKELLSCSHPFSKQAVEHVQSHLSKKQVTSTLFQPYIEEICESLRGSIFQKFMESDKFTRFCQWK
NVELNIHLTMNDFSVHRIIGRGGFGEVYGCRKADTGKMYAMKCLDKKRIKMKQGETLALNERIMLSLVSTGDCPFIVCMTYAFHTPDKL
CFILDLMNGGDLHYHLSQHGVFSEKEMRFYATEIILGLEHMHNRFVVYRDLKPANILLDEHGHVRISDLGLACDFSKKKPHASVGTHGY
MAPEVLQKGTAYDSSADWFSLGCMLFKLLRGHSPFRQHKTKDKHEIDRMTLTMNVELPDVFSPELKSLLEGLLQRDVSKRLGCHGGSAQ
ELKTHDFFRGIDWQHVYLQKYPPPLIPPRGEVNAADAFDIGSFDEEDTKGIKLLDCDQELYKNFPLVISERWQQEVAETVYEAVNADTD
KIEARKRAKNKQLGHEEDYALGRDCIVHGYMLKLGNPFLTQWQRRYFYLFPNRLEWRGEGESRQSLLTMEQIVSVEETQIKDKKCILLR
IKGGKQFVLQCESDPEFVQWKKELTETFMEAQRLLRRAPKFLNKSRSAVVELSKPPLCHRNSNGL

SEQ ID NO: 27

ATGGCGGACCTGGAGGCCGTGCTGGCCGATGTCAGCTACCTGATGGCGATGGAGAAGAGCAAGGCGACCCCGGCCGCCCGCGCCAGCAA
GAAGATCGTCCTGCCCGAGCCCAGTATCCGGAGCGTGATGCAGAAGTATCTTGAGGAGAGACACGAAATCACCTTTGACAAGATTTTTA
ATCAGAGAATTGGTTTCTTGCTATTTAAAGATTTTTGTTTGAATGAAATTAATGAAGCTGTACCTCAGGTGAAGTTTTATGAAGAGATA
AAAGAATATGAAAAGCTTGAGAATGAGGAAGATCGCCTTTGTAGAAGTCGACAGATTTATGACACTTACATCATGAAGGAGCTGCTGTC
GTGTTCACATCCATTCTCAAAGCAAGCCGTAGAACACGTACAAAGTCATCTGTCCAAGAAACAAGTGACATCAACTCTTTTTCAGCCAT
ACATAGAAGAAATTTGTGAAAGTCTCCGAGGCAGCATTTTTCAAAAATTCATGGAAAGTGACAAGTTTACTAGATTTTGTCAGTGGAAA
AACGTGGAATTAAATATCCATTTGACCATGAATGATTTCAGCGTGCATCGGATCATTGGACGAGGAGGATTCGGTGAAGTATACGGTTG
CAGGAAAGCAGACACTGGAAAGATGTATGCAATGAAATGCTTGGATAAGAAGAGAATCAAGATGAAACAGGGAGAAACCTTAGCCTTAA
ATGAAAGGATCATGTTGTCCCTGGTGAGCACAGGAGATTGCCCTTTCATCGTCTGTATGACCTATGCCTTCCACACTCCAGATAAACTG
TGCTTCATCTTGGATCTGATGAACGGGGGTGACCTGCACTATCACCTTTCGCAGCACGGGGTGTTTTCTGAGAAGGAGATGCGGTTTTA
CGCCACAGAAATCATCCTGGGGCTGGAACACATGCACAATCGGTTTGTTGTTTACAGAGACTTGAAGCCCGCCAATATCCTCCTGGATG
AGCACGGACATGTGAGGATATCAGACCTTGGTCTTGCCTGCGATTTTTCCAAAAAGAAGCCGCACGCGAGCGTGGGCACCCACGGGTAC
ATGGCGCCCGAAGTTCTGCAGAAGGGGACCGCCTACGACAGCAGTGCCGACTGGTTCTCCCTGGGCTGTATGCTTTTCAAACTTCTGAG
AGGTCACAGCCCTTTCAGACAACATAAAACCAAAGATAAGCATGAGATAGACCGAATGACTCTCACCATGAACGTGGAACTTCCAGACG
TCTTCTCCCCTGAGCTCAAGTCCCTTCTGGAAGGCCTGCTTCAGCGAGATGTCAGTAAGCGCCTCGGCTGCCATGGAGGCAGCGCACAG
GAGCTAAAAACGCACGACTTCTTCAGAGGCATCGACTGGCAGCACGTCTACCTGCAGAAGTACCCTCCACCCTTGATCCCTCCCCGAGG
GGAAGTCAATGCAGCCGACGCCTTTGACATCGGCTCATTTGATGAAGAGGATACCAAAGGCATCAAGCTTCTTGATTGCGACCAAGAAC
TCTACAAGAACTTCCCTCTGGTGATCTCTGAGCGCTGGCAGCAGGAAGTGGCGGAAACAGTTTATGAAGCAGTAAATGCAGACACGGAT
AAAATCGAGGCCAGGAAGAGAGCTAAAAATAAGCAGCTTGGCCACGAAGAAGATTACGCCCTGGGAAGAGACTGCATCGTGCACGGGTA
CATGCTGAAGCTGGGGAACCCTTTCCTGACCCAGTGGCAGCGCCGCTATTTTTACCTCTTTCCGAACAGACTTGAGTGGAGAGGAGAAG
GCGAGTCGCGACAAAGTTTACTGACAATGGAACAGATTGTGTCCGTGGAAGAAACTCAGATTAAAGACAAAAAGTGCATTTTGTTGAGA
ATAAAAGGAGGGAAGCAGTTCGTTTTGCAGTGTGAGAGTGACCCAGAGTTTGTGCAGTGGAAGAAAGAGCTGACGGAGACATTCATGGA
GGCCCAGCGGCTGCTACGGCGAGCCCCCAAGTTCCTCAACAAATCCCGCTCAGCCGTCGTGGAACTCTCAAAGCCTCCCCTCTGCCATA
GGAACAGCAACGGCCTCTGA

SEQ ID NO: 28

Human GRK2 (G protein-coupled receptor kinase 2) (NM_001619)
Adrenergic, beta, receptor kinase 1(ADRBK1)
(Beta-adrenergic receptor kinase 1 (Bark-1):

MADLEAVLADVSYLMAMEKSKATPAARASKKILLPEPSIRSVMQKYLEDRGEVTFEKIFSQKLGYLLFRDFCLNHLEEARPLVEFYEEI
KKYEKLETEEERVARSREIFDSYIMKELLACSHPFSKSATEHVQGHLGKKQVPPDLFQPYIEEICQNLRGDVFQKFIESDKFTRFCQWK
NVELNIHLTMNDFSVHRIIGRGGFGEVYGCRKADTGKMYAMKCLDKKRIKMKQGETLALNERIMLSLVSTGDCPFIVCMSYAFHTPDKL
SFILDLMNGGDLHYHLSQHGVFSEADMRFYAAEIILGLEHMHNRFVVYRDLKPANILLDEHGHVRISDLGLACDFSKKKPHASVGTHGY
MAPEVLQKGVAYDSSADWFSLGCMLFKLLRGHSPFRQHKTKDKHEIDRMTLTMAVELPDSFSPELRSLLEGLLQRDVNRRLGCLGRGAQ
EVKESPFFRSLDWQMVFLQKYPPPLIPPRGEVNAADAFDIGSFDEEDTKGIKLLDSDQELYRNFPLTISERWQQEVAETVFDTINAETD
RLEARKKAKNKQLGHEEDYALGKDCIMHGYMSKMGNPFLTQWQRRYFYLFPNRLEWRGEGEAPQSLLTMEEIQSVEETQIKERKCLLLK
IRGGKQFILQCDSDPELVQWKKELRDAYREAQQLVQRVPKMKNKPRSPVVELSKVPLVQRGSANGL

SEQ ID NO: 29

ATGGCGGACCTGGAGGCGGTGCTGGCCGACGTGAGCTACCTGATGGCCATGGAGAAGAGCAAGGCCACGCCGGCCGCGCGCGCCAGCAA
GAAGATACTGCTGCCCGAGCCCAGCATCCGCAGTGTCATGCAGAAGTACCTGGAGGACCGGGGCGAGGTGACCTTTGAGAAGATCTTTT
CCCAGAAGCTGGGGTACCTGCTCTTCCGAGACTTCTGCCTGAACCACCTGGAGGAGGCCAGGCCCTTGGTGGAATTCTATGAGGAGATC
AAGAAGTACGAGAAGCTGGAGACGGAGGAGGAGCGTGTGGCCCGCAGCCGGGAGATCTTCGACTCATACATCATGAAGGAGCTGCTGGC
CTGCTCGCATCCCTTCTCGAAGAGTGCCACTGAGCATGTCCAAGGCCACCTGGGGAAGAAGCAGGTGCCTCCGGATCTCTTCCAGCCAT
ACATCGAAGAGATTTGTCAAAACCTCCGAGGGGACGTGTTCCAGAAATTCATTGAGAGCGATAAGTTCACACGGTTTTGCCAGTGGAAG
AATGTGGAGCTCAACATCCACCTGACCATGAATGACTTCAGCGTGCATCGCATCATTGGGCGCGGGGGCTTTGGCGAGGTCTATGGGTG
CCGGAAGGCTGACACAGGCAAGATGTACGCCATGAAGTGCCTGGACAAAAAGCGCATCAAGATGAAGCAGGGGGAGACCCTGGCCCTGA
ACGAGCGCATCATGCTCTCGCTCGTCAGCACTGGGGACTGCCCATTCATTGTCTGCATGTCATACGCGTTCCACACGCCAGACAAGCTC
AGCTTCATCCTGGACCTCATGAACGGTGGGGACCTGCACTACCACCTCTCCCAGCACGGGGTCTTCTCAGAGGCTGACATGCGCTTCTA
TGCGGCCGAGATCATCCTGGGCCTGGAGCACATGCACAACCGCTTCGTGGTCTACCGGGACCTGAAGCCAGCCAACATCCTTCTGGACG
AGCATGGCCACGTGCGGATCTCGGACCTGGGCCTGGCCTGTGACTTCTCCAAGAAGAAGCCCCATGCCAGCGTGGGCACCCACGGGTAC
ATGGCTCCGGAGGTCCTGCAGAAGGGCGTGGCCTACGACAGCAGTGCCGACTGGTTCTCTCTGGGGTGCATGCTCTTCAAGTTGCTGCG
GGGGCACAGCCCCTTCCGGCAGCACAAGACCAAAGACAAGCATGAGATCGACCGCATGACGCTGACGATGGCCGTGGAGCTGCCCGACT
CCTTCTCCCCTGAACTACGCTCCCTGCTGGAGGGGTTGCTGCAGAGGGATGTCAACCGGAGATTGGGCTGCCTGGGCCGAGGGGCTCAG
GAGGTGAAAGAGAGCCCCTTTTTCCGCTCCCTGGACTGGCAGATGGTCTTCTTGCAGAAGTACCCTCCCCCGCTGATCCCCCCACGAGG
GGAGGTGAACGCGGCCGACGCCTTCGACATTGGCTCCTTCGATGAGGAGGACACAAAAGGAATCAAGTTACTGGACAGTGATCAGGAGC
TCTACCGCAACTTCCCCCTCACCATCTCGGAGCGGTGGCAGCAGGAGGTGGCAGAGACTGTCTTCGACACCATCAACGCTGAGACAGAC
CGGCTGGAGGCTCGCAAGAAAGCCAAGAACAAGCAGCTGGGCCATGAGGAAGACTACGCCCTGGGCAAGGACTGCATCATGCATGGCTA
CATGTCCAAGATGGGCAACCCCTTCCTGACCCAGTGGCAGCGGCGGTACTTCTACCTGTTCCCCAACCGCCTCGAGTGGCGGGGCGAGG
GCGAGGCCCCGCAGAGCCTGCTGACCATGGAGGAGATCCAGTCGGTGGAGGAGACGCAGATCAAGGAGCGCAAGTGCCTGCTCCTCAAG
ATCCGCGGTGGGAAACAGTTCATTTTGCAGTGCGATAGCGACCCTGAGCTGGTGCAGTGGAAGAAGGAGCTGCGCGACGCCTACCGCGA
GGCCCAGCAGCTGGTGCAGCGGGTGCCCAAGATGAAGAACAAGCCGCGCTCGCCCGTGGTGGAGCTGAGCAAGGTGCCGCTGGTCCAGC
GCGGCAGTGCCAACGGCCTCTGA

SEQ ID NO: 30

Human GRK2 (C20)(G protein-coupled receptor kinase 2) (NM_001619)
Adrenergic, beta, receptor kinase 1(ADRBK1)
(Beta-adrenergic receptor kinase 1 (Bark-1):

MADLEAVLADVSYLMAMEKSKATPAARASKKILLPEPSIRSVMQKYLEDRGEVTFEKIFSQKLGYLLFRDFCLNHLEEARPLVEFYEEI
KKYEKLETEEERVARSREIFDSYIMKELLACSHPFSKSATEHVQGHLGKKQVPPDLFQPYIEEICQNLRGDVFQKFIESDKFTRFCQWK
NVELNIHLTMNDFSVHRIIGRGGFGEVYGCRKADTGKMYAMKCLDKKRIKMKQGETLALNERIMLSLVSTGDCPFIVCMSYAFHTPDKL
SFILDLMNGGDLHYHLSQHGVFSEADMRFYAAEIILGLEHMHNRFVVYRDLKPANILLDEHGHVRISDLGLACDFSKKKPHASVGTHGY
MAPEVLQKGVAYDSSADWFSLGCMLFKLLRGHSPFRQHKTKDKHEIDRMTLTMAVELPDSFSPELRSLLEGLLQRDVNRRLGCLGRGAQ
EVKESPFFRSLDWQMVFLQKYPPPLIPPRGEVNAADAFDIGSFDEEDTKGIKLLDSDQELYRNFPLTISERWQQEVAETVFDTINAETD
RLEARKKAKNKQLGHEEDYALGKDCIMHGYMSKMGNPFLTQWQRRYFYLFPNRLEWRGEGEAPQSLLTMEEIQSVEETQIKERKCLLLK
IRGGKQFILQCDSDPELVQWKKELRDAYREAQQLVQRVPKMKNKPRSPVVELSKVPLVQRGSCVLL

SEQ ID NO: 31

ATGGCGGACCTGGAGGCGGTGCTGGCCGACGTGAGCTACCTGATGGCCATGGAGAAGAGCAAGGCCACGCCGGCCGCGCGCGCCAGCAA
GAAGATACTGCTGCCCGAGCCCAGCATCCGCAGTGTCATGCAGAAGTACCTGGAGGACCGGGGCGAGGTGACCTTTGAGAAGATCTTTT
CCCAGAAGCTGGGGTACCTGCTCTTCCGAGACTTCTGCCTGAACCACCTGGAGGAGGCCAGGCCCTTGGTGGAATTCTATGAGGAGATC
AAGAAGTACGAGAAGCTGGAGACGGAGGAGGAGCGTGTGGCCCGCAGCCGGGAGATCTTCGACTCATACATCATGAAGGAGCTGCTGGC
CTGCTCGCATCCCTTCTCGAAGAGTGCCACTGAGCATGTCCAAGGCCACCTGGGGAAGAAGCAGGTGCCTCCGGATCTCTTCCAGCCAT
ACATCGAAGAGATTTGTCAAAACCTCCGAGGGGACGTGTTCCAGAAATTCATTGAGAGCGATAAGTTCACACGGTTTTGCCAGTGGAAG
AATGTGGAGCTCAACATCCACCTGACCATGAATGACTTCAGCGTGCATCGCATCATTGGGCGCGGGGGCTTTGGCGAGGTCTATGGGTG
CCGGAAGGCTGACACAGGCAAGATGTACGCCATGAAGTGCCTGGACAAAAAGCGCATCAAGATGAAGCAGGGGGAGACCCTGGCCCTGA
ACGAGCGCATCATGCTCTCGCTCGTCAGCACTGGGGACTGCCCATTCATTGTCTGCATGTCATACGCGTTCCACACGCCAGACAAGCTC
AGCTTCATCCTGGACCTCATGAACGGTGGGGACCTGCACTACCACCTCTCCCAGCACGGGGTCTTCTCAGAGGCTGACATGCGCTTCTA
TGCGGCCGAGATCATCCTGGGCCTGGAGCACATGCACAACCGCTTCGTGGTCTACCGGGACCTGAAGCCAGCCAACATCCTTCTGGACG
AGCATGGCCACGTGCGGATCTCGGACCTGGGCCTGGCCTGTGACTTCTCCAAGAAGAAGCCCCATGCCAGCGTGGGCACCCACGGGTAC
ATGGCTCCGGAGGTCCTGCAGAAGGGCGTGGCCTACGACAGCAGTGCCGACTGGTTCTCTCTGGGGTGCATGCTCTTCAAGTTGCTGCG
GGGGCACAGCCCCTTCCGGCAGCACAAGACCAAAGACAAGCATGAGATCGACCGCATGACGCTGACGATGGCCGTGGAGCTGCCCGACT
CCTTCTCCCCTGAACTACGCTCCCTGCTGGAGGGGTTGCTGCAGAGGGATGTCAACCGGAGATTGGGCTGCCTGGGCCGAGGGGCTCAG
GAGGTGAAAGAGAGCCCCTTTTTCCGCTCCCTGGACTGGCAGATGGTCTTCTTGCAGAAGTACCCTCCCCCGCTGATCCCCCCACGAGG
GGAGGTGAACGCGGCCGACGCCTTCGACATTGGCTCCTTCGATGAGGAGGACACAAAAGGAATCAAGTTACTGGACAGTGATCAGGAGC
TCTACCGCAACTTCCCCCTCACCATCTCGGAGCGGTGGCAGCAGGAGGTGGCAGAGACTGTCTTCGACACCATCAACGCTGAGACAGAC
CGGCTGGAGGCTCGCAAGAAAGCCAAGAACAAGCAGCTGGGCCATGAGGAAGACTACGCCCTGGGCAAGGACTGCATCATGCATGGCTA
CATGTCCAAGATGGGCAACCCCTTCCTGACCCAGTGGCAGCGGCGGTACTTCTACCTGTTCCCCAACCGCCTCGAGTGGCGGGGCGAGG
GCGAGGCCCCGCAGAGCCTGCTGACCATGGAGGAGATCCAGTCGGTGGAGGAGACGCAGATCAAGGAGCGCAAGTGCCTGCTCCTCAAG
ATCCGCGGTGGGAAACAGTTCATTTTGCAGTGCGATAGCGACCCTGAGCTGGTGCAGTGGAAGAAGGAGCTGCGCGACGCCTACCGCGA
GGCCCAGCAGCTGGTGCAGCGGGTGCCCAAGATGAAGAACAAGCCGCGCTCGCCCGTGGTGGAGCTGAGCAAGGTGCCGCTGGTCCAGC
GCGGCAGTTGTGTGCTTCTTTAG

SEQ ID NO: 32

Bovine GRK2 (G protein-coupled receptor kinase 2) (M34019.1)
Bovine beta-adrenergic receptor kinase (beta-ARK):

MADLEAVLADVSYLMAMEKSKATPAARASKKILLPEPSIRSVMQKYLEDRGEVTFEKIFSQKLGYLLFRDFCLKHLEEAKPLVEFYEEI
KKYEKLETEEERLVCSREIFDTYIMKELLACSHPFSKSAIEHVQGHLVKKQVPPDLFQPYIEEICQNLRGDVFQKFIESDKFTRFCQWK
NVELNIHLTMNDFSVHRIIGRGGFGEVYGCRKADTGKMYAMKCLDKKRIKMKQGETLALNERIMLSLVSTGDCPFIVCMSYAFHTPDKL
SFILDLMNGGDLHYHLSQHGVFSEADMRFYAAEIILGLEHMHNRFVVYRDLKPANILLDEHGHVRISDLGLACDFSKKKPHASVGTHGY
MAPEVLQKGVAYDSSADWFSLGCMLFKLLRGHSPFRQHKTKDKHEIDRMTLTMAVELPDSFSPELRSLLEGLLQRDVNRRLGCLGRGAQ
EVKESPFFRSLDWQMVFLQKYPPPLIPPRGEVNAADAFDIGSFDEEDTKGIKLLDSDQELYRNFPLTISERWQQEVAETVFDTINAETD
RLEARKKTKNKQLGHEEDYALGKDCIMHGYMSKMGNPFLTQWQRRYFYLFPNRLEWRGEGEAPQSLLTMEEIQSVEETQIKERKCLLLK
IRGGKQFVLQCDSDPELVQWKKELRDAYREAQQLVQRVPKMKNKPRSPVVELSKVPLIQRGSANGL

SEQ ID NO: 33

ATGGCGGACCTGGAGGCGGTGCTGGCCGACGTGAGCTACCTGATGGCCATGGAGAAGAGCAAGGCCACGCCGGCGGCGCGCGCCAGCAA
GAAGATCCTGCTGCCCGAGCCCAGCATCCGCAGCGTCATGCAGAAGTACCTGGAGGACCGGGGCGAGGTGACTTTTGAGAAGATCTTCT
CCCAGAAGCTGGGGTACCTGCTTTTCCGAGACTTCTGCCTGAAGCACCTGGAGGAGGCCAAGCCCTTGGTAGAGTTCTACGAGGAGATC
AAGAAATACGAGAAGCTGGAGACAGAGGAGGAGCGCCTGGTCTGCAGCCGAGAGATCTTCGACACGTACATCATGAAGGAGCTGCTGGC
CTGCTCACATCCTTTCTCGAAGAGCGCCATTGAGCACGTCCAGGGCCATCTGGTGAAGAAGCAGGTGCCTCCGGATCTCTTCCAGCCAT
ATATTGAAGAAATTTGCCAGAACCTCCGAGGAGACGTGTTCCAGAAATTCATCGAGAGCGATAAATTCACACGATTTTGCCAGTGGAAG
AATGTAGAGCTCAACATCCACCTGACCATGAACGACTTCAGTGTGCACCGCATCATCGGGCGAGGCGGCTTCGGTGAGGTCTACGGCTG
CCGGAAGGCCGACACGGGCAAGATGTACGCCATGAAGTGTCTGGACAAGAAGCGCATCAAGATGAAGCAAGGGGAGACTCTGGCCCTGA
ATGAGCGCATCATGCTGTCGCTCGTCAGCACCGGGGACTGCCCGTTCATCGTCTGCATGTCATACGCCTTCCACACACCGGACAAGCTC
AGCTTCATCCTGGATCTCATGAACGGCGGGGACCTGCACTACCACCTGTCCCAGCACGGGGTCTTCTCCGAGGCCGACATGCGTTTCTA
CGCCGCCGAGATCATCCTGGGCCTGGAGCACATGCACAACCGCTTCGTGGTCTACCGGGACCTGAAGCCGGCCAACATCCTGCTGGACG
AGCACGGCCACGTGCGCATCTCAGACCTGGGCCTGGCCTGTGACTTCTCCAAGAAGAAGCCTCACGCCAGTGTGGGCACCCACGGGTAC
ATGGCTCCCGAGGTTCTACAGAAGGGTGTGGCCTACGACAGCAGCGCCGACTGGTTCTCCCTGGGCTGCATGCTCTTCAAGCTGCTGCG
AGGGCATAGCCCTTTCCGGCAGCACAAGACCAAAGACAAGCATGAGATCGACAGAATGACATTGACAATGGCTGTGGAGCTGCCTGACT
CCTTCTCCCCTGAGCTCCGCTCCTTGCTGGAGGGGCTGCTGCAGAGGGATGTCAACCGGAGGCTAGGCTGCCTGGGCCGAGGGGCCCAG
GAGGTGAAGGAGAGCCCCTTCTTCCGTTCCCTGGACTGGCAGATGGTCTTTTTACAAAAGTACCCTCCCCCGTTGATCCCCCCACGAGG
GGAGGTGAATGCAGCCGACGCCTTTGACATTGGCTCCTTCGATGAGGAGGACACAAAAGGAATCAAGCTACTGGACAGTGACCAGGAGC
TCTACCGCAACTTCCCCCTGACCATCTCGGAGCGGTGGCAGCAGGAGGTAGCAGAGACTGTCTTTGACACCATCAATGCTGAGACGGAC
CGGCTGGAGGCCCGCAAGAAAACCAAAAACAAGCAGTTGGGCCACGAGGAAGACTACGCCCTGGGCAAGGACTGCATCATGCATGGCTA
CATGTCCAAGATGGGCAACCCCTTCCTGACCCAGTGGCAGCGGCGGTACTTCTACCTGTTCCCTAACCGGCTCGAGTGGCGGGGCGAGG
GCGAGGCCCCGCAGAGCCTGCTGACCATGGAGGAGATCCAGTCGGTGGAGGAGACGCAGATCAAGGAGCGAAAGTGCCTCCTCCTCAAG
ATCCGAGGTGGCAAGCAGTTTGTCCTGCAGTGCGATAGTGACCCAGAGCTGGTGCAGTGGAAGAAGGAGCTTCGAGACGCCTACCGCGA
GGCCCAGCAGCTAGTGCAGCGGGTGCCCAAGATGAAGAACAAGCCGCGCTCGCCCGTCGTGGAGCTGAGCAAGGTGCCACTGATCCAGC
GCGGCAGTGCCAACGGCCTCTGA

SEQ ID NO: 34

Fig. 3

Amino Acid sequence of wild-type hGPR3 ACCESSION NP_005272

MMWGAGSPLAWLSAGSGNVNVSSVGPAEGPTGPAAPLPSPKAWDVVLCISGTLVSCENALVVAIIVGTPAFRAPMFLLVG
SLAVADLLAGLGLVLHFAAVFCIGSAEMSLVLVGVLAMAFTASIGSLLAITVDRYLSLYNALTYYSETTVTRTYVMLALV
WGGALGLGLLPVLAWNCLDGLTTCGVVYPLSKNHLVVLAIAFFMVFGIMLQLYAQICRIVCRHAQQIALQRHLLPASHYV
ATRKGIATLAVVLGAFAACWLPFTVYCLLGDAHSPPLYTYLTLLPATYNSMINPIIYAFRNQDVQKVLWAVCCCCSSSKI
PFRSRSPSDV

SEQ ID No:35

Nucleotide sequence of wild-type hGPR3 ACCESSION NM_ 005281

ATGATGTGGGGTGCAGGCAGCCCTCTGGCCTGGCTCTCAGCTGGCTCAGGCAACGTGAATGTAAGCAGCGTGGGCCCAGC
AGAGGGGCCCACAGGTCCAGCCGCACCACTGCCCTCGCCTAAGGCCTGGGATGTGGTGCTCTGCATCTCAGGCACCCTGG
TGTCCTGCGAGAATGCGCTAGTGGTGGCCATCATCGTGGGCACTCCTGCCTTCCGTGCCCCCATGTTCCTGCTGGTGGGC
AGCCTGGCCGTGGCAGACCTGCTGGCAGGCCTGGGCCTGGTCCTGCACTTTGCTGCTGTCTTCTGCATCGGCTCAGCGGA
GATGAGCCTGGTGCTGGTTGGCGTGCTGGCAATGGCCTTTACCGCCAGCATCGGCAGTCTACTGGCCATCACTGTCGACC
GCTACCTTTCTCTGTACAATGCCCTCACCTACTATTCAGAGACAACAGTGACACGGACCTATGTGATGCTGGCCTTAGTG
TGGGGAGGTGCCCTGGGCCTGGGGCTGCTGCCTGTGCTGGCCTGGAACTGCCTGGATGGCCTGACCACATGTGGCGTGGT
TTATCCACTCTCCAAGAACCATCTGGTAGTTCTGGCCATTGCCTTCTTCATGGTGTTTGGCATCATGCTGCAGCTCTACG
CCCAAATCTGCCGCATCGTCTGCCGCCATGCCCAGCAGATTGCCCTTCAGCGGCACCTGCTGCCTGCCTCCCACTATGTG
GCCACCCGCAAGGGCATTGCCACACTGGCCGTGGTGCTTGGAGCCTTTGCCGCCTGCTGGTTGCCCTTCACTGTCTACTG
CCTGCTGGGTGATGCCCACTCTCCACCTCTCTACACCTATCTTACCTTGCTCCCTGCCACCTACAACTCCATGATCAACC
CTATCATCTACGCCTTCCGCAACCAGGATGTGCAGAAAGTGCTGTGGGCTGTCTGCTGCTGCTGTTCCTCTTCCAAGATC
CCCTTCCGATCCCGCTCCCCCAGTGATGTCTAG

SEQ ID No:36

Amino Acid sequence of HA tagged hGPR3

MYPYDVPDYAAAAAMMWGAGSPLAWLSAGSGNVNVSSVGPAEGPTGPAAPLPSPKAWDVVLCISGTLVSCENALVVAIIVG
TPAFRAPMFLLVGSLAVADLLAGLGLVLHFAAVFCIGSAEMSLVLVGVLAMAFTASIGSLLAITVDRYLSLYNALTYYSET
TVTRTYVMLALVWGGALGLGLLPVLAWNCLDGLTTCGVVYPLSKNHLVVLAIAFFMVFGIMLQLYAQICRIVCRHAQQIAL
QRHLLPASHYVATRKGIATLAVVLGAFAACWLPFTVYCLLGDAHSPPLYTYLTLLPATYNSMINPIIYAFRNQDVQKVLWA
VCCCCSSSKIPFRSRSPSDV

SEQ ID No:37

Nucleotide sequence of HA tagged hGPR3

ATGTACCCATACGACGTACCTGATTACGCAGCAGCAGCAGCAATGATGTGGGGTGCAGGCAGCCCTCTGGCCTGGCTCTCAG
CTGGCTCAGGCAACGTGAATGTAAGCAGCGTGGGCCCAGCAGAGGGGCCCACAGGTCCAGCCGCACCACTGCCCTCGCCTAA
GGCCTGGGATGTGGTGCTCTGCATCTCAGGCACCCTGGTGTCCTGCGAGAATGCGCTAGTGGTGGCCATCATCGTGGGCACT
CCTGCCTTCCGTGCCCCCATGTTCCTGCTGGTGGGCAGCCTGGCCGTGGCAGACCTGCTGGCAGGCCTGGGCCTGGTCCTGC
ACTTTGCTGCTGTCTTCTGCATCGGCTCAGCGGAGATGAGCCTGGTGCTGGTTGGCGTGCTGGCAATGGCCTTTACCGCCAG
CATCGGCAGTCTACTGGCCATCACTGTCGACCGCTACCTTTCTCTGTACAATGCCCTCACCTACTATTCAGAGACAACAGTG
ACACGGACCTATGTGATGCTGGCCTTAGTGTGGGGAGGTGCCCTGGGCCTGGGGCTGCTGCCTGTGCTGGCCTGGAACTGCC
TGGATGGCCTGACCACATGTGGCGTGGTTTATCCACTCTCCAAGAACCATCTGGTAGTTCTGGCCATTGCCTTCTTCATGGT
GTTTGGCATCATGCTGCAGCTCTACGCCCAAATCTGCCGCATCGTCTGCCGCCATGCCCAGCAGATTGCCCTTCAGCGGCAC
CTGCTGCCTGCCTCCCACTATGTGGCCACCCGCAAGGGCATTGCCACACTGGCCGTGGTGCTTGGAGCCTTTGCCGCCTGCT
GGTTGCCCTTCACTGTCTACTGCCTGCTGGGTGATGCCCACTCTCCACCTCTCTACACCTATCTTACCTTGCTCCCTGCCAC
CTACAACTCCATGATCAACCCTATCATCTACGCCTTCCGCAACCAGGATGTGCAGAAAGTGCTGTGGGCTGTCTGCTGCTGC
TGTTCCTCTTCCAAGATCCCCTTCCGATCCCGCTCCCCCAGTGATGTCTAG

SEQ ID No:38

Amino Acid sequence of the hGPR3- Enhanced Receptor

MMWGAGSPLAWLSAGSGNVNVSSVGPAEGPTGPAAPLPSPKAWDVVLCISGTLVSCENALVVAIIVGTPAFRAPMFLLVG
SLAVADLLAGLGLVLHFAAVFCIGSAEMSLVLVGVLAMAFTASIGSLLAITVDRYLSLYNALTYYSETTVTRTYVMLALV
WGGALGLGLLPVLAWNCLDGLTTCGVVYPLSKNHLVVLAIAFFMVFGIMLQLYAQICRIVCRHAQQIALQRHLLPASHYV
ATRKGIATLAVVLGAFAACWLPFTVYCLLGDAHSPPLYTYLTLLPATYNSMINPIIYAFRNQDVQKVLWAVCCCCAAARG
RTPPSLGPQDESCTTASSSLAKDTSS

SEQ ID No:39

Nucleotide sequence of the hGPR3- Enhanced Receptor

ATGATGTGGGGTGCAGGCAGCCCTCTGGCCTGGCTCTCAGCTGGCTCAGGCAACGTGAATGTAAGCAGCGTGGGCCCAGC
AGAGGGGCCCACAGGTCCAGCCGCACCACTGCCCTCGCCTAAGGCCTGGGATGTGGTGCTCTGCATCTCAGGCACCCTGG
TGTCCTGCGAGAATGCGCTAGTGGTGGCCATCATCGTGGGCACTCCTGCCTTCCGTGCCCCCATGTTCCTGCTGGTGGGC
AGCCTGGCCGTGGCAGACCTGCTGGCAGGCCTGGGCCTGGTCCTGCACTTTGCTGCTGTCTTCTGCATCGGCTCAGCGGA
GATGAGCCTGGTGCTGGTTGGCGTGCTGGCAATGGCCTTTACYGCCAGCATCGGCAGTCTACTGGCCATCACTGTCGACC
GCTACCTTTCTCTGTACAATGCCCTCACCTACTATTCAGAGACAACAGTGACACGGACCTATGTGATGCTGGCCTTAGTG
TGGGGAGGTGCCCTGGGCCTGGGGCTGCTGCCTGTGCTGGCCTGGAACTGCCTGGATGGCCTGACCACATGTGGCGTGGT
TTATCCACTCTCCAAGAACCATCTGGTAGTTCTGGCCATTGCCTTCTTCATGGTGTTTGGCATCATGCTGCAGCTCTACG
CCCAAATCTGCCGCATCGTCTGCCGCCATGCCCAGCAGATTGCCCTTCAGCGGCACCTGCTGCCTGCCTCCCACTATGTG
GCCACCCGCAAGGGCATTGCCACACTGGCCGTGGTGCTTGGAGCCTTTGCCGCCTGCTGGTTGCCCTTCACTGTCTACTG
CCTGCTGGGTGATGCCCACTCTCCACCTCTCTACACCTATCTTACCTTGCTCCCTGCCACCTACAACTCCATGATCAACC
CTATCATCTACGCCTTCCGCAACCAGGATGTGCAGAAAGTGCTGTGGGCTGTCTGCTGCTGCTGTGCGGCCGCACGGGGA
CGCACCCCACCCAGCCTGGGTCCCCAAGATGAGTCCTGCACCACCGCCAGcTCCTCCCTGGCCAAGGACACTTCATCGTG
A

SEQ ID No:40

Amino Acid sequence of the HA tagged hGPR3- Enhanced Receptor

MYPYDVPDYAAAAAMMWGAGSPLAWLSAGSGNVNVSSVGPAEGPTGPAAPLPSPKAWDVVLCISGTLVSCENALVVAIIV
GTPAFRAPMFLLVGSLAVADLLAGLGLVLHFAAVFCIGSAEMSLVLVGVLAMAFTASIGSLLAITVDRYLSLYNALTYYS
ETTVTRTYVMLALVWGGALGLGLLPVLAWNCLDGLTTCGVVYPLSKNHLVVLAIAFFMVFGIMLQLYAQICRIVCRHAQQ
IALQRHLLPASHYVATRKGIATLAVVLGAFAACWLPFTVYCLLGDAHSPPLYTYLTLLPATYNSMINPIIYAFRNQDVQK
VLWAVCCCCAAARGRTPPSLGPQDESCTTASSSLAKDTSS

SEQ ID No:41

Nucleotide sequence of the HA tagged hGPR3- Enhanced Receptor

ATGTACCCATACGACGTACCTGATTACGCAGCAGCAGCAGCAATGATGTGGGGTGCAGGCAGCCCTCTGGCCTGGCTCTC
AGCTGGCTCAGGCAACGTGAATGTAAGCAGCGTGGGCCCAGCAGAGGGGCCCACAGGTCCAGCCGCACCACTGCCCTCGC
CTAAGGCCTGGGATGTGGTGCTCTGCATCTCAGGCACCCTGGTGTCCTGCGAGAATGCGCTAGTGGTGGCCATCATCGTG
GGCACTCCTGCCTTCCGTGCCCCCATGTTCCTGCTGGTGGGCAGCCTGGCCGTGGCAGACCTGCTGGCAGGCCTGGGCCT
GGTCCTGCACTTTGCTGCTGTCTTCTGCATCGGCTCAGCGGAGATGAGCCTGGTGCTGGTTGGCGTGCTGGCAATGGCCT
TTACTGCCAGCATCGGCAGTCTACTGGCCATCACTGTCGACCGCTACCTTTCTCTGTACAATGCCCTCACCTACTATTCA
GAGACAACAGTGACACGGACCTATGTGATGCTGGCCTTAGTGTGGGGAGGTGCCCTGGGCCTGGGGCTGCTGCCTGTGCT
GGCCTGGAACTGCCTGGATGGCCTGACCACATGTGGCGTGGTTTATCCACTCTCCAAGAACCATCTGGTAGTTCTGGCCA
TTGCCTTCTTCATGGTGTTTGGCATCATGCTGCAGCTCTACGCCCAAATCTGCCGCATCGTCTGCCGCCATGCCCAGCAG
ATTGCCCTTCAGCGGCACCTGCTGCCTGCCTCCCACTATGTGGCCACCCGCAAGGGCATTGCCACACTGGCCGTGGTGCT
TGGAGCCTTTGCCGCCTGCTGGTTGCCCTTCACTGTCTACTGCCTGCTGGGTGATGCCCACTCTCCACCTCTCTACACCT
ATCTTACCTTGCTCCCTGCCACCTACAACTCCATGATCAACCCTATCATCTACGCCTTCCGCAACCAGGATGTGCAGAAA
GTGCTGTGGGCTGTCTGCTGCTGCTGTGCGGCCGCACGGGGACGCACCCCACCCAGCCTGGGTCCCCAAGATGAGTCCTG
CACCACCGCCAGCTCCTCCCTGGCCAAGGACACTTCATCGTGA

SEQ ID No:42

Amino Acid sequence of the wild-type hGPR6 ACCESSION NP_005275

MNASAASLNDSQVVVVAAEGAAAAATAAGGPDTGEWGPPAAAALGAGGGANGSLELSSQLSAGPPGLLLPAVNPWDVLLC
VSGTVIAGENALVVALIASTPALRTPMFVLVGSLATADLLAGCGLILHFVFQYLVPSETVSLLTVGFLVASFAASVSSLL
AITVDRYLSLYNALTYYSRRTLLGVHLLLAATWTVSLGLGLLPVLGWNCLAERAACSVVRPLARSHVALLSAAFFMVFGI
MLHLYVRICQVVWRHAHQIALQQHCLAPPHLAATRKGVGTLAVVLGTFGASWLPFAIYCVVGSHEDPAVYTYATLLPATY
NSMINPIIYAFRNQEIQRALWLLLCGCFQSKVPFRSRSPSEV

SEQ ID No:43

Nucleotide sequence of the wild-type hGPR6 ACCESSION NM_ 005284

ATGAACGCGAGCGCCGCCTCGCTCAACGACTCCCAGGTGGTGGTAGTGGCGGCCGAAGGAGCGGCGGCGGCGGCCACAGC
AGCAGGGGGGCCGGACACGGGCGAATGGGGACCCCCTGCTGCGGCGGCTCTAGGAGCCGGCGGCGGAGCTAATGGGTCTC
TGGAGCTGTCCTCGCAGCTGTCGGCTGGGCCACCGGGACTCCTGCTGCCAGCGGTGAATCCGTGGGACGTGCTCCTGTGC
GTGTCGGGGACAGTGATCGCTGGAGAAAACGCGCTGGTGGTGGCGCTCATCGCGTCCACTCCGGCGCTGCGCACGCCCAT
GTTCGTGCTGGTAGGCAGCCTGGCCACCGCTGACCTGTTGGCGGGCTGTGGCCTCATCTTGCACTTTGTGTTCCAGTACT
TGGTGCCCTCGGAGACTGTGAGTCTGCTCACGGTGGGCTTCCTCGTGGCCTCCTTCGCCGCCTCTGTCAGCAGCCTGCTG
GCCATTACGGTGGACCGCTACCTGTCCCTGTATAACGCGCTCACCTATTACTCGCGCCGGACCCTGTTGGGCGTGCACCT
CCTGCTTGCCGCCACTTGGACCGTGTCCCTAGGCCTGGGGCTGCTGCCCGTGCTGGGCTGGAACTGCCTGGCAGAGCGCG
CCGCCTGCAGCGTGGTGCGCCCGCTGGCGCGCAGCCACGTGGCTCTGCTCTCCGCCGCCTTCTTCATGGTCTTCGGCATC
ATGCTGCACCTGTACGTGCGCATCTGCCAGGTGGTCTGGCGCCACGCGCACCAGATCGCGCTGCAGCAGCACTGCCTGGC
GCCACCCCATCTCGCTGCCACCAGAAAGGGTGTGGGTACACTGGCTGTGGTGCTGGGCACTTTCGGCGCCAGCTGGCTGC
CCTTCGCCATCTATTGCGTGGTGGGCAGCCATGAGGACCCGGCGGTCTACACTTACGCCACCCTGCTGCCCGCCACCTAC
AACTCCATGATCAATCCCATCATCTATGCCTTCCGCAACCAGGAGATCCAGCGCGCCCTGTGGCTCCTGCTCTGTGGCTG
TTTCCAGTCCAAAGTGCCCTTTCGTTCCAGGTCTCCCAGCGAGGTCTGA

SEQ ID No:44

Amino Acid sequence of the HA tagged wild-type hGPR6

MYPYDVPDYAAAAAMNASAASLNDSQVVVVAAEGAAAAATAAGGPDTGEWGPPAAAALGAGGGANGSLELSSQLSAGPPGL
LLPAVNPWDVLLCVSGTVIAGENALVVALIASTPALRTPMFVLVGSLATADLLAGCGLILHFVFQYLVPSETVSLLTVGFL
VASFAASVSSLLAITVDRYLSLYNALTYYSRRTLLGVHLLLAATWTVSLGLGLLPVLGWNCLAERAACSVVRPLARSHVAL
LSAAFFMVFGIMLHLYVRICQVVWRHAHQIALQQHCLAPPHLAATRKGVGTLAVVLGTFGASWLPFAIYCVVGSHEDPAVY
TYATLLPATYNSMINPIIYAFRNQEIQRALWLLLCGCFQSKVPFRSRSPSEV

SEQ ID No:45

Nucleotide sequence of the HA tagged wild-type hGPR6

ATGTACCCATACGACGTACCTGATTACGCAGCAGCAGCAGCAATGAACGCGAGCGCCGCCTCGCTCAACGACTCCCAGGTGGT
GGTAGTGGCGGCCGAAGGAGCGGCGGCGGCGGCCACAGCAGCAGGGGGGCCGGACACGGGCGAATGGGGACCCCCTGCTGCGG
CGGCTCTAGGAGCCGGCGGCGGAGCTAATGGGTCTCTGGAGCTGTCCTCGCAGCTGTCGGCTGGGCCACCGGGACTCCTGCTG
CCAGCGGTGAATCCGTGGGACGTGCTCCTGTGCGTGTCGGGGACAGTGATCGCTGGAGAAAACGCGCTGGTGGTGGCGCTCAT
CGCGTCCACTCCGGCGCTGCGCACGCCCATGTTCGTGCTGGTAGGCAGCCTGGCCACCGCTGACCTGTTGGCGGGCTGTGGCC
TCATCTTGCACTTTGTGTTCCAGTACTTGGTGCCCTCGGAGACTGTGAGTCTGCTCACGGTGGGCTTCCTCGTGGCCTCCTTC
GCCGCCTCTGTCAGCAGCCTGCTGGCCATTACGGTGGACCGCTACCTGTCCCTGTATAACGCGCTCACCTATTACTCGCGCCG
GACCCTGTTGGGCGTGCACCTCCTGCTTGCCGCCACTTGGACCGTGTCCCTAGGCCTGGGGCTGCTGCCCGTGCTGGGCTGGA
ACTGCCTGGCAGAGCGCGCCGCCTGCAGCGTGGTGCGCCCGCTGGCGCGCAGCCACGTGGCTCTGCTCTCCGCCGCCTTCTTC
ATGGTCTTCGGCATCATGCTGCACCTGTACGTGCGCATCTGCCAGGTGGTCTGGCGCCACGCGCACCAGATCGCGCTGCAGCA
GCACTGCCTGGCGCCACCCCATCTCGCTGCCACCAGAAAGGGTGTGGGTACACTGGCTGTGGTGCTGGGCACTTTCGGCGCCA
GCTGGCTGCCCTTCGCCATCTATTGCGTGGTGGGCAGCCATGAGGACCCGGCGGTCTACACTTACGCCACCCTGCTGCCCGCC
ACCTACAACTCCATGATCAATCCCATCATCTATGCCTTCCGCAACCAGGAGATCCAGCGCGCCCTGTGGCTCCTGCTCTGTGG
CTGTTTCCAGTCCAAAGTGCCCTTTCGTTCCAGGTCTCCCAGCGAGGTCTGA

SEQ ID No:46

Amino Acid sequence of the hGPR6- Enhanced Receptor

MNASAASLNDSQVVVVAAEGAAAAATAAGGPDTGEWGPPAAAALGAGGGANGSLELSSQLSAGPPGLLLPAVNPWDVLLC
VSGTVIAGENALVVALIASTPALRTPMFVLVGSLATADLLAGCGLILHFVFQYLVPSETVSLLTVGFLVASFAASVSSLL
AITVDRYLSLYNALTYYSRRTLLGVHLLLAATWTVSLGLGLLPVLGWNCLAERAACSVVRPLARSHVALLSAAFFMVFGI
MLHLYVRICQVVWRHAHQIALQQHCLAPPHLAATRKGVGTLAVVLGTFGASWLPFAIYCVVGSHEDPAVYTYATLLPATY
NSMINPIIYAFRNQEIQRALWLLLCGCAAARGRTPPSLGPQDESCTTASSSLAKDTSS

SEQ ID No:47

Nucleotide sequence of the hGPR6- Enhanced Receptor

ATGAACGCGAGCGCCGCCTCGCTCAACGACTCCCAGGTGGTGGTAGTGGCGGCCGAAGGAGCGGCGGCGGCGGCCACAGC
AGCAGGGGGGCCGGACACGGGCGAATGGGGACCCCCTGCTGCGGCGGCTCTAGGAGCCGGCGGCGGAGCTAATGGGTCTC
TGGAGCTGTCCTCGCAGCTGTCGGCTGGGCCACCGGGACTCCTGCTGCCAGCGGTGAATCCGTGGGACGTGCTCCTGTGC
GTGTCGGGGACAGTGATCGCTGGAGAAAACGCGCTGGTGGTGGCGCTCATCGCGTCCACTCCGGCGCTGCGCACGCCCAT
GTTCGTGCTGGTAGGCAGCCTGGCCACCGCTGACCTGTTGGCGGGCTGTGGCCTCATCTTGCACTTTGTGTTCCAGTACT
TGGTGCCCTCGGAGACTGTGAGTCTGCTCACGGTGGGCTTCCTCGTGGCCTCCTTCGCCGCCTCTGTCAGCAGCCTGCTG
GCCATTACGGTGGACCGCTACCTGTCCCTGTATAACGCGCTCACCTATTACTCGCGCCGGACCCTGTTGGGCGTGCACCT
CCTGCTTGCCGCCACTTGGACCGTGTCCCTAGGCCTGGGGCTGCTGCCCGTGCTGGGCTGGAACTGCCTGGCAGAGCGCG
CCGCCTGCAGCGTGGTGCGCCCGCTGGCGCGCAGCCACGTGGCTCTGCTCTCCGCCGCCTTCTTCATGGTCTTCGGCATC
ATGCTGCACCTGTACGTGCGCATCTGCCAGGTGGTCTGGCGCCACGCGCACCAGATCGCGCTGCAGCAGCACTGCCTGGC
GCCACCCCATCTCGCTGCCACCAGAAAGGGTGTGGGTACACTGGCTGTGGTGCTGGGCACTTTCGGCGCCAGCTGGCTGC
CCTTCGCCATCTATTGCGTGGTGGGCAGCCATGAGGACCCGGCGGTCTACACTTACGCCACCCTGCTGCCCGCCACCTAC
AACTCCATGATCAATCCCATCATCTATGCCTTCCGCAACCAGGAGATCCAGCGCGCCCTGTGGCTCCTGCTCTGTGGCTG
TGCGGCCGCACGGGGACGCACCCCACCCAGCCTGGGTCCCCAAGATGAGTCCTGCACCACCGCCAGCTCCTCCCTGGCCA
AGGACACTTCATCGTGA

SEQ ID No:48

Amino Acid sequence of the HA tagged hGPR6- Enhanced Receptor

MYPYDVPDYAAAAAMNASAASLNDSQVVVVAAEGAAAAATAAGGPDTGEWGPPAAAALGAGGGANGSLELSSQLSAGPPG
LLLPAVNPWDVLLCVSGTVIAGENALVVALIASTPALRTPMFVLVGSLATADLLAGCGLILHFVFQYLVPSETVSLLTVG
FLVASFAASVSSLLAITVDRYLSLYNALTYYSRRTLLGVHLLLAATWTVSLGLGLLPVLGWNCLAERAACSVVRPLARSH
VALLSAAFFMVFGIMLHLYVRICQVVWRHAHQIALQQHCLAPPHLAATRKGVGTLAVVLGTFGASWLPFAIYCVVGSHED
PAVYTYATLLPATYNSMINPIIYAFRNQEIQRALWLLLCGCAAARGRTPPSLGPQDESCTTASSSLAKDTSS

SEQ ID No:49

Nucleotide sequence of the HA tagged hGPR6- Enhanced Receptor

ATGTACCCATACGACGTACCTGATTACGCAGCAGCAGCAGCAATGAACGCGAGCGCCGCCTCGCTCAACGACTCCCAGGT
GGTGGTAGTGGCGGCCGAAGGAGCGGCGGCGGCGGCCACAGCAGCAGGGGGGCCGGACACGGGCGAATGGGGACCCCCTG
CTGCGGCGGCTCTAGGAGCCGGCGGCGGAGCTAATGGGTCTCTGGAGCTGTCCTCGCAGCTGTCGGCTGGGCCACCGGGA
CTCCTGCTGCCAGCGGTGAATCCGTGGGACGTGCTCCTGTGCGTGTCGGGGACAGTGATCGCTGGAGAAAACGCGCTGGT
GGTGGCGCTCATCGCGTCCACTCCGGCGCTGCGCACGCCCATGTTCGTGCTGGTAGGCAGCCTGGCCACCGCTGACCTGT
TGGCGGGCTGTGGCCTCATCTTGCACTTTGTGTTCCAGTACTTGGTGCCCTCGGAGACTGTGAGTCTGCTCACGGTGGGC
TTCCTCGTGGCCTCCTTCGCCGCCTCTGTCAGCAGCCTGCTGGCCATTACGGTGGACCGCTACCTGTCCCTGTATAACGC
GCTCACCTATTACTCGCGCCGGACCCTGTTGGGCGTGCACCTCCTGCTTGCCGCCACTTGGACCGTGTCCCTAGGCCTGG
GGCTGCTGCCCGTGCTGGGCTGGAACTGCCTGGCAGAGCGCGCCGCCTGCAGCGTGGTGCGCCCGCTGGCGCGCAGCCAC
GTGGCTCTGCTCTCCGCCGCCTTCTTCATGGTCTTCGGCATCATGCTGCACCTGTACGTGCGCATCTGCCAGGTGGTCTG
GCGCCACGCGCACCAGATCGCGCTGCAGCAGCACTGCCTGGCGCCACCCCATCTCGCTGCCACCAGAAAGGGTGTGGGTA
CACTGGCTGTGGTGCTGGGCACTTTCGGCGCCAGCTGGCTGCCCTTCGCCATCTATTGCGTGGTGGGCAGCCATGAGGAC
CCGGCGGTCTACACTTACGCCACCCTGCTGCCCGCCACCTACAACTCCATGATCAATCCCATCATCTATGCCTTCCGCAA
CCAGGAGATCCAGCGCGCCCTGTGGCTCCTGCTCTGTGGCTGTGCGGCCGCACGGGGACGCACCCCACCCAGCCTGGGTC
CCCAAGATGAGTCCTGCACCACCGCCAGCTCCTCCCTGGCCAAGGACACTTCATCGTGA

SEQ ID No:50

Amino Acid sequence of the wild-type hGPR12 ACCESSION NP_005279

MNEDLKVNLSGLPRDYLDAAAAENISAAVSSRVPAVEPEPELVVNPWDIVLCTSGTLISCENAIVVLIIFHNPSLRAPMF
LLIGSLALADLLAGIGLITNFVFAYLLQSEATKLVTIGLIVASFSASVCSLLAITVDRYLSLYYALTYHSERTVTFTYVM
LVMLWGTSICLGLLPVMGWNCLRDESTCSVVRPLTKNNAAILSVSFLFMFALMLQLYIQICKIVMRHAHQIALQHHFLAT
SHYVTTRKGVSTLAIILGTFAACWMPFTLYSLIADYTYPSIYTYATLLPATYNSIINPVIYAFRNQEIQKALCLICCGCI
PSSLAQRARSPSDV

SEQ ID No:51

Nucleotide sequence of the wild-type hGPR12 ACCESSION NM_005288

ATGAATGAAGACCTGAAGGTCAATTTAAGCGGGCTGCCTCGGGATTATTTAGATGCCGCTGCTGCGGAGAACATCTCGGC
TGCTGTCTCCTCCCGGGTTCCTGCCGTAGAGCCAGAGCCTGAGCTCGTAGTCAACCCCTGGGACATTGTCTTGTGTACCT
CGGGAACCCTCATCTCCTGTGAAAATGCCATTGTGGTCCTTATCATCTTCCACAACCCCAGCCTGCGAGCACCCATGTTC
CTGCTAATAGGCAGCCTGGCTCTTGCAGACCTGCTGGCCGGCATTGGACTCATCACCAATTTTGTTTTTGCCTACCTGCT
TCAGTCAGAAGCCACCAAGCTGGTCACGATCGGCCTCATTGTCGCCTCTTTCTCTGCCTCTGTCTGCAGCTTGCTGGCTA
TCACTGTTGACCGCTACCTCTCACTGTACTACGCTCTGACGTACCATTCGGAGAGGACGGTCACGTTTACCTATGTCATG
CTCGTCATGCTCTGGGGGACCTCCATCTGCCTGGGGCTGCTGCCCGTCATGGGCTGGAACTGCCTCCGAGACGAGTCCAC
CTGCAGCGTGGTCAGACCGCTCACCAAGAACAACGCGGCCATCCTCTCGGTGTCCTTCCTCTTCATGTTTGCGCTCATGC
TTCAGCTCTACATCCAGATCTGTAAGATTGTGATGAGGCACGCCCATCAGATAGCCCTGCAGCACCACTTCCTGGCCACG
TCGCACTATGTGACCACCCGGAAAGGGGTCTCCACCCTGGCTATCATCCTGGGGACGTTTGCTGCTTGCTGGATGCCTTT
CACCCTCTATTCCTTGATAGCGGATTACACCTACCCCTCCATCTATACCTACGCCACCCTCCTGCCCGCCACCTACAATT
CCATCATCAACCCTGTCATATATGCTTTCAGAAACCAAGAGATCCAGAAAGCGCTCTGTCTCATTTGCTGCGGCTGCATC
CCGTCCAGTCTCGCCCAGAGAGCGCGCTCGCCCAGTGATGTGTAG

SEQ ID No:52

Amino Acid sequence of the HA tagged wild-type hGPR12

MYPYDVPDYAAAAAMNEDLKVNLSGLPRDYLDAAAAENISAAVSSRVPAVEPEPELVVNPWDIVLCTSGTLISCENAIVVLI
IFHNPSLRAPMFLLIGSLALADLLAGIGLITNFVFAYLLQSEATKLVTIGLIVASFSASVCSLLAITVDRYLSLYYALTYHS
ERTVTFTYVMLVMLWGTSICLGLLPVMGWNCLRDESTCSVVRPLTKNNAAILSVSFLFMFALMLQLYIQICKIVMRHAHQIA
LQHHFLATSHYVTTRKGVSTLAIILGTFAACWMPFTLYSLIADYTYPSIYTYATLLPATYNSIINPVIYAFRNQEIQKALCL
ICCGCIPSSLAQRARSPSDV

SEQ ID No:53

Nucleotide sequence of the HA tagged wild-type hGPR12

ATGTACCCATACGACGTACCTGATTACGCAGCAGCAGCAGCAATGAATGAAGACCTGAAGGTCAATTTAAGCGGGCTGCCT
CGGGATTATTTAGATGCCGCTGCTGCGGAGAACATCTCGGCTGCTGTCTCCTCCCGGGTTCCTGCCGTAGAGCCAGAGCCT
GAGCTCGTAGTCAACCCCTGGGACATTGTCTTGTGTACCTCGGGAACCCTCATCTCCTGTGAAAATGCCATTGTGGTCCTT
ATCATCTTCCACAACCCCAGCCTGCGAGCACCCATGTTCCTGCTAATAGGCAGCCTGGCTCTTGCAGACCTGCTGGCCGGC
ATTGGACTCATCACCAATTTTGTTTTTGCCTACCTGCTTCAGTCAGAAGCCACCAAGCTGGTCACGATCGGCCTCATTGTC
GCCTCTTTCTCTGCCTCTGTCTGCAGCTTGCTGGCTATCACTGTTGACCGCTACCTCTCACTGTACTACGCTCTGACGTAC
CATTCGGAGAGGACGGTCACGTTTACCTATGTCATGCTCGTCATGCTCTGGGGGACCTCCATCTGCCTGGGGCTGCTGCCC
GTCATGGGCTGGAACTGCCTCCGAGACGAGTCCACCTGCAGCGTGGTCAGACCGCTCACCAAGAACAACGCGGCCATCCTC
TCGGTGTCCTTCCTCTTCATGTTTGCGCTCATGCTTCAGCTCTACATCCAGATCTGTAAGATTGTGATGAGGCACGCCCAT
CAGATAGCCCTGCAGCACCACTTCCTGGCCACGTCGCACTATGTGACCACCCGGAAAGGGGTCTCCACCCTGGCTATCATC
CTGGGGACGTTTGCTGCTTGCTGGATGCCTTTCACCCTCTATTCCTTGATAGCGGATTACACCTACCCCTCCATCTATACC
TACGCCACCCTCCTGCCCGCCACCTACAATTCCATCATCAACCCTGTCATATATGCTTTCAGAAACCAAGAGATCCAGAAA
GCGCTCTGTCTCATTTGCTGCGGCTGCATCCCGTCCAGTCTCGCCCAGAGAGCGCGCTCGCCCAGTGATGTGTAG

SEQ ID No:54

Amino Acid sequence of the hGPR12- Enhanced Receptor

MNEDLKVNLSGLPRDYLDAAAAENISAAVSSRVPAVEPEPELVVNPWDIVLCTSGTLISCENAIVVLIIFHNPSLRAPMF
LLIGSLALADLLAGIGLITNFVFAYLLQSEATKLVTIGLIVASFSASVCSLLAITVDRYLSLYYALTYHSERTVTFTYVM
LVMLWGTSICLGLLPVMGWNCLRDESTCSVVRPLTKNNAAILSVSFLFMFALMLQLYIQICKIVMRHAHQIALQHHFLAT
SHYVTTRKGVSTLAIILGTFAACWMPFTLYSLIADYTYPSIYTYATLLPATYNSIINPVIYAFRNQEIQKALCLICCGCA
AARGRTPPSLGPQDESCTTASSSLAKDTSS

SEQ ID No:55

Nucleotide sequence of the hGPR12- Enhanced Receptor

ATGAATGAAGACCTGAAGGTCAATTTAAGCGGGCTGCCTCGGGATTATTTAGATGCCGCTGCTGCGGAGAACATCTCGGC
TGCTGTCTCCTCCCGGGTTCCTGCCGTAGAGCCAGAGCCTGAGCTCGTAGTCAACCCCTGGGACATTGTCTTGTGTACCT
CGGGAACCCTCATCTCCTGTGAAAATGCCATTGTGGTCCTTATCATCTTCCACAACCCCAGCCTGCGAGCACCCATGTTC
CTGCTAATAGGCAGCCTGGCTCTTGCAGACCTGCTGGCCGGCATTGGACTCATCACCAATTTTGTTTTTGCCTACCTGCT
TCAGTCAGAAGCCACCAAGCTGGTCACGATCGGCCTCATTGTCGCCTCTTTCTCTGCCTCTGTCTGCAGCTTGCTGGCTA
TCACTGTTGACCGCTACCTCTCACTGTACTACGCTCTGACGTACCATTCGGAGAGGACGGTCACGTTTACCTATGTCATG
CTCGTCATGCTCTGGGGGACCTCCATCTGCCTGGGGCTGCTGCCCGTCATGGGCTGGAACTGCCTCCGAGACGAGTCCAC
CTGCAGCGTGGTCAGACCGCTCACCAAGAACAACGCGGCCATCCTCTCGGTGTCCTTCCTCTTCATGTTTGCGCTCATGC
TTCAGCTCTACATCCAGATCTGTAAGATTGTGATGAGGCACGCCCATCAGATAGCCCTGCAGCACCACTTCCTGGCCACG
TCGCACTATGTGACCACCCGGAAAGGGGTCTCCACCCTGGCTATCATCCTGGGGACGTTTGCTGCTTGCTGGATGCCTTT
CACCCTCTATTCCTTGATAGCGGATTACACCTACCCCTCCATCTATACCTACGCCACCCTCCTGCCCGCCACCTACAATT
CCATCATCAACCCTGTCATATATGCTTTCAGAAACCAAGAGATCCAGAAAGCGCTCTGTCTCATTTGCTGCGGCTGCGCG
GCCGCACGGGGACGCACCCCACCCAGCCTGGGTCCCCAAGATGAGTCCTGCACCACCGCCAGCTCCTCCCTGGCCAAGGA
CACTTCATCGTGA

SEQ ID No:56

Amino Acid sequence of the HA tagged hGPR12- Enhanced Receptor

MYPYDVPDYAAAAAMNEDLKVNLSGLPRDYLDAAAAENISAAVSSRVPAVEPEPELVVNPWDIVLCTSGTLISCENAIVV
LIIFHNPSLRAPMFLLIGSLALADLLAGIGLITNFVFAYLLQSEATKLVTIGLIVASFSASVCSLLAITVDRYLSLYYAL
TYHSERTVTFTYVMLVMLWGTSICLGLLPVMGWNCLRDESTCSVVRPLTKNNAAILSVSFLFMFALMLQLYIQICKIVMR
HAHQIALQHHFLATSHYVTTRKGVSTLAIILGTFAACWMPFTLYSLIADYTYPSIYTYATLLPATYNSIINPVIYAFRNQ
EIQKALCLICCGCAAARGRTPPSLGPQDESCTTASSSLAKDTSS

SEQ ID No:57

Nucleotide sequence of the HA tagged hGPR12-Enhanced Receptor

ATGTACCCATACGACGTACCTGATTACGCAGCAGCAGCAGCAATGAATGAAGACCTGAAGGTCAATTTAAGCGGGCTGCC
TCGGGATTATTTAGATGCCGCTGCTGCGGAGAACATCTCGGCTGCTGTCTCCTCCCGGGTTCCTGCCGTAGAGCCAGAGC
CTGAGCTCGTAGtCAACCCCTGGGACATTgTCTTGTGTACCTCGGGAACCCTCATCTCCTGTGAAAATGCCATTGTGGTC
CTTATCATCTTCCACAACCCCAGCCTGCGAGCACCCATGTTCCTGCTAATAGGCAGCCTGGCTCTTGCAGACCTGCTGGC
CGGCATTGGACTCATCACCAATTTTGTTTTTGCCTACCTGCTTCAGTCAGAAGCCACCAAGCTGGTCACGATCGGCCTCA
TTGTCGCCTCTTTCTCTGCCTCTGTCTGCAGCTTGCTGGCTATCACTGTTGACCGCTACCTCTCACTGTACTACGCTCTG
ACGTACCATTCGGAGAGGACGGTCACGTTTACCTATGTCATGCTCGTCATGCTCTGGGGGACCTCCATCTGCCTGGGGCT
GCTGCCCGTCATGGGCTGGAACTGCCTCCGAGACGAGTCCACCTGCAGCGTGGTCAGACCGCTCACCAAGAACAACGCGG
CCATCCTCTCGGTGTCCTTCCTCTTCATGTTTGCGCTCATGCTTCAGCTCTACATCCAGATCTGTAAGATTGTGATGAGG
CACGCCCATCAGATAGCCCTGCAGCACCACTTCCTGGCCACGTCGCACTATGTGACCACCCGGAAAGGGGTCTCCACCCT
GGCTATCATCCTGGGGACGTTTGCTGCTTGCTGGATGCCTTTCACCCTCTATTCCTTGATAGCGGATTACACCTACCCCT
CCATCTATACCtACGCCACCCTCCTGCCCGCCACCTACAATTCCATCATCAACCCTGTCATATATGCTTTCAGAAACCAA
GAGATCCAGAAAGCGCTCTGTCTCATTTGCTGCGGCTGCGCGGCCGCACGGGGACGCACCCCACCCAGCCTGGGTCCCCA
AGATGAGTCCTGCACCACCGCCAGCTCCTCCCTGGCCAAGGACACTTCATCGTGA

SEQ ID No:58

Amino Acid sequence of the wild-type hSREB3 ACCESSION NP_061842

MANTTGEPEEVSGALSPPSASAYVKLVLLGLIMCVSLAGNAILSLLVLKERALHKAPYYFLLDLCLADGIRSAVCFPFVL
ASVRHGSSWTFSALSCKIVAFMAVLFCFHAAFMLFCISVTRYMAIAHHRFYAKRMTLWTCAAVICMAWTLSVAMAFPPVF
DVGTYKFIREEDQCIFEHRYFKANDTLGFMLMLAVLMAATHAVYGKLLLFEYRHRKMKPVQMVPAISQNWTFHGPGATGQ
AAANWIAGFGRGPMPPTLLGIRQNGHAASRRLLGMDEVKGEKQLGRMFYAITLLFLLLWSPYIVACYWRVFVKACAVPHR
YLATAVWMSFAQAAVNPIVCFLLNKDLKKCLRTHAPCWGTGGAPAPREPYCVM

SEQ ID No:59

Nucleotide sequence of the wild-type hSREB3 ACCESSION NM_018969

ATGGCCAACACTACCGGAGAGCCTGAGGAGGTGAGCGGCGCTCTGTCCCCACCGTCCGCATCAGCTTATGTGAAGCTGGT
ACTGCTGGGACTGATTATGTGCGTGAGCCTGGCGGGTAACGCCATCTTGTCCCTGCTGGTGCTCAAGGAGCGTGCCCTGC
ACAAGGCTCCTTACTACTTCCTGCTGGACCTGTGCCTGGCCGATGGCATACGCTCTGCCGTCTGCTTCCCCTTTGTGCTG
GCTTCTGTGCGCCACGGCTCTTCATGGACCTTCAGTGCACTCAGCTGCAAGATTGTGGCCTTTATGGCCGTGCTCTTTTG
CTTCCATGCGGCCTTCATGCTGTTCTGCATCAGCGTCACCCGCTACATGGCCATCGCCCACCACCGCTTCTACGCCAAGC
GCATGACACTCTGGACATGCGCGGCTGTCATCTGCATGGCCTGGACCCTGTCTGTGGCCATGGCCTTCCCACCTGTCTTT
GACGTGGGCACCTACAAGTTTATTCGGGAGGAGGACCAGTGCATCTTTGAGCATCGCTACTTCAAGGCCAATGACACGCT
GGGCTTCATGCTTATGTTGGCTGTGCTCATGGCAGCTACCCATGCTGTCTACGGCAAGCTGCTCCTCTTCGAGTATCGTC
ACCGCAAGATGAAGCCAGTGCAGATGGTGCCAGCCATCAGCCAGAACTGGACATTCCATGGTCCCGGGGCCACCGGCCAG
GCTGCTGCCAACTGGATCGCCGGCTTTGGCCGTGGGCCCATGCCACCAACCCTGCTGGGTATCCGGCAGAATGGGCATGC
AGCCAGCCGGCGGCTACTGGGCATGGACGAGGTCAAGGGTGAAAAGCAGCTGGGCCGCATGTTCTACGCGATCACACTGC
TCTTTCTGCTCCTCTGGTCACCCTACATCGTGGCCTGCTACTGGCGAGTGTTTGTGAAAGCCTGTGCTGTGCCCCACCGC
TACCTGGCCACTGCTGTTTGGATGAGCTTCGCCCAGGCTGCCGTCAACCCAATTGTCTGCTTCCTGCTCAACAAGGACCT
CAAGAAGTGCCTGAGGACTCACGCCCCCTGCTGGGGCACAGGAGGTGCCCCGGCTCCCAGAGAACCCTACTGTGTCATGT
GA

SEQ ID No:60

Amino Acid sequence of the HA tagged wild-type hSREB3

MYPYDVPDYAAAAAMANTTGEPEEVSGALSPPSASAYVKLVLLGLIMCVSLAGNAILSLLVLKERALHKAPYYFLLDLCLADGIRS
AVCFPFVLASVRHGSSWTFSALSCKIVAFMAVLFCFHAAFMLFCISVTRYMAIAHHRFYAKRMTLWTCAAVICMAWTLSVAMAFPP
VFDVGTYKFIREEDQCIFEHRYFKANDTLGFMLMLAVLMAATHAVYGKLLLFEYRHRKMKPVQMVPAISQNWTFHGPGATGQAAAN
WIAGFGRGPMPPTLLGIRQNGHAASRRLLGMDEVKGEKQLGRMFYAITLLFLLLWSPYIVACYWRVFVKACAVPHRYLATAVWMSF
AQAAVNPIVCFLLNKDLKKCLRTHAPCWGTGGAPAPREPYCVM

SEQ ID No:61

Nucleotide sequence of the HA tagged wild-type hSREB3

ATGTACCCATACGACGTACCTGATTACGCAGCAGCAGCAGCAATGGCCAACACTACCGGAGAGCCTGAGGAGGTGAGCGG
CGCTCTGTCCCCACCGTCCGCATCAGCTTATGTGAAGCTGGTACTGCTGGGACTGATTATGTGCGTGAGCCTGGCGGGTA
ACGCCATCTTGTCCCTGCTGGTGCTCAAGGAGCGTGCCCTGCACAAGGCTCCTTACTACTTCCTGCTGGACCTGTGCCTG
GCCGATGGCATACGCTCTGCCGTCTGCTTCCCCTTTGTGCTGGCTTCTGTGCGCCACGGCTCTTCATGGACCTTCAGTGC
ACTCAGCTGCAAGATTGTGGCCTTTATGGCCGTGCTCTTTTGCTTCCATGCGGCCTTCATGCTGTTCTGCATCAGCGTCA
CCCGCTACATGGCCATCGCCCACCACCGCTTCTACGCCAAGCGCATGACACTCTGGACATGCGCGGCTGTCATCTGCATG
GCCTGGACCCTGTCTGTGGCCATGGCCTTCCCACCTGTCTTTGACGTGGGCACCTACAAGTTTATTCGGGAGGAGGACCA
GTGCATCTTTGAGCATCGCTACTTCAAGGCCAATGACACGCTGGGCTTCATGCTTATGTTGGCTGTGCTCATGGCAGCTA
CCCATGCTGTCTACGGCAAGCTGCTCCTCTTCGAGTATCGTCACCGCAAGATGAAGCCAGTGCAGATGGTGCCAGCCATC
AGCCAGAACTGGACATTCCATGGTCCCGGGGCCACCGGCCAGGCTGCTGCCAACTGGATCGCCGGCTTTGGCCGTGGGCC
CATGCCACCAACCCTGCTGGGTATCCGGCAGAATGGGCATGCAGCCAGCCGGCGGCTACTGGGCATGGACGAGGTCAAGG
GTGAAAAGCAGCTGGGCCGCATGTTCTACGCGATCACACTGCTCTTTCTGCTCCTCTGGTCACCCTACATCGTGGCCTGC
TACTGGCGAGTGTTTGTGAAAGCCTGTGCTGTGCCCCACCGCTACCTGGCCACTGCTGTTTGGATGAGCTTCGCCCAGGC
TGCCGTCAACCCAATTGTCTGCTTCCTGCTCAACAAGGACCTCAAGAAGTGCCTGAGGACTCACGCCCCCTGCTGGGGCA
CAGGAGGTGCCCCGGCTCCCAGAGAACCCTACTGTGTCATGTGA

SEQ ID No:62

Amino Acid sequence of the hSREB3- Enhanced Receptor

MANTTGEPEEVSGALSPPSASAYVKLVLLGLIMCVSLAGNAILSLLVLKERALHKAPYYFLLDLCLADGIRSAVCFPFVL
ASVRHGSSWTFSALSCKIVAFMAVLFCFHAAFMLFCISVTRYMAIAHHRFYAKRMTLWTCAAVICMAWTLSVAMAFPPVF
DVGTYKFIREEDQCIFEHRYFKANDTLGFMLMLAVLMAATHAVYGKLLLFEYRHRKMKPVQMVPAISQNWTFHGPGATGQ
AAANWIAGFGRGPMPPTLLGIRQNGHAASRRLLGMDEVKGEKQLGRMFYAITLLFLLLWSPYIVACYWRVFVKACAVPHR
YLATAVWMSFAQAAVNPIVCFLLNKDLKKCLRTHAPCAAARGRTPPSLGPQDESCTTASSSLAKDTSS

SEQ ID No:63

Nucleotide sequence of the hSREB3- Enhanced Receptor

ATGGCCAACACTACCGGAGAGCCTGAGGAGGTGAGCGGCGCTCTGTCCCCACCGTCCGCATCAGCTTATGTGAAGCTGGT
ACTGCTGGGACTGATTATGTGCGTGAGCCTGGCGGGTAACGCCATCTTGTCCCTGCTGGTGCTCAAGGAGCGTGCCCTGC
ACAAGGCTCCTTACTACTTCCTGCTGGACCTGTGCCTGGCCGATGGCATACGCTCTGCCGTCTGCTTCCCCTTTGTGCTG
GCTTCTGTGCGCCACGGCTCTTCATGGACCTTCAGTGCACTCAGCTGCAAGATTGTGGCCTTTATGGCCGTGCTCTTTTG
CTTCCATGCGGCCTTCATGCTGTTCTGCATCAGCGTCACCCGCTACATGGCCATCGCCCACCACCGCTTCTACGCCAAGC
GCATGACACTCTGGACATGCGCGGCTGTCATCTGCATGGCCTGGACCCTGTCTGTGGCCATGGCCTTCCCACCTGTCTTT
GACGTGGGCACCTACAAGTTTATTCGGGAGGAGGACCAGTGCATCTTTGAGCATCGCTACTTCAAGGCCAATGACACGCT
GGGCTTCATGCTTATGTTGGCTGTGCTCATGGCAGCTACCCATGCTGTCTACGGCAAGCTGCTCCTCTTCGAGTATCGTC
ACCGCAAGATGAAGCCAGTGCAGATGGTGCCAGCCATCAGCCAGAACTGGACATTCCATGGTCCCGGGGCCACCGGCCAG
GCTGCTGCCAACTGGATCGCCGGCTTTGGCCGTGGGCCCATGCCACCAACCCTGCTGGGTATCCGGCAGAATGGGCATGC
AGCCAGCCGGCGGCTACTGGGCATGGACGAGGTCAAGGGTGAAAAGCAGCTGGGCCGCATGTTCTACGCGATCACACTGC
TCTTTCTGCTCCTCTGGTCACCCTACATCGTGGCCTGCTACTGGCGAGTGTTTGTGAAAGCCTGTGCTGTGCCCCACCGC
TACCTGGCCACTGCTGTTTGGATGAGCTTCGCCCAGGCTGCCGTCAACCCAATTGTCTGCTTCCTGCTCAACAAGGACCT
CAAGAAGTGCCTGAGGACTCACGCCCCCTGCGCGGCCGCACGGGGACGCACCCCACCCAGCCTGGGTCCCCAAGATGAGT
CCTGCACCACCGCCAGCTCCTCCCTGGCCAAGGACACTTCATCGTGA

SEQ ID No:64

Amino Acid sequence of the HA tagged hSREB3- Enhanced Receptor

MYPYDVPDYAAAAAMANTTGEPEEVSGALSPPSASAYVKLVLLGLIMCVSLAGNAILSLLVLKERALHKAPYYFLLDLCLA
DGIRSAVCFPFVLASVRHGSSWTFSALSCKIVAFMAVLFCFHAAFMLFCISVTRYMAIAHHRFYAKRMTLWTCAAVICMAW
TLSVAMAFPPVFDVGTYKFIREEDQCIFEHRYFKANDTLGFMLMLAVLMAATHAVYGKLLLFEYRHRKMKPVQMVPAISQN
WTFHGPGATGQAAANWIAGFGRGPMPPTLLGIRQNGHAASRRLLGMDEVKGEKQLGRMFYAITLLFLLLWSPYIVACYWRV
FVKACAVPHRYLATAVWMSFAQAAVNPIVCFLLNKDLKKCLRTHAPCAAARGRTPPSLGPQDESCTTASSSLAKDTSS

SEQ ID No:65

Nucleotide sequence of the HA tagged hSREB3- Enhanced Receptor

ATGTACCCATACGACGTACCTGATTACGCAGCAGCAGCAGCAATGGCCAACACTACCGGAGAGCCTGAGGAGGTGAGCGGCG
CTCTGTCCCCACCGTCCGCATCAGCTTATGTGAAGCTGGTACTGCTGGGACTGATTATGTGCGTGAGCCTGGCGGGTAACGC
CATCTTGTCCCTGCTGGTGCTCAAGGAGCGTGCCCTGCACAAGGCTCCTTACTACTTCCTGCTGGACCTGTGCCTGGCCGAT
GGCATACGCTCTGCCGTCTGCTTCCCCTTTGTGCTGGCTTCTGTGCGCCACGGCTCTTCATGGACCTTCAGTGCACTCAGCT
GCAAGATTGTGGCCTTTATGGCCGTGCTCTTTTGCTTCCATGCGGCCTTCATGCTGTTCTGCATCAGCGTCACCCGCTACAT
GGCCATCGCCCACCACCGCTTCTACGCCAAGCGCATGACACTCTGGACATGCGCGGCTGTCATCTGCATGGCCTGGACCCTG
TCTGTGGCCATGGCCTTCCCACCTGTCTTTGACGTGGGCACCTACAAGTTTATTCGGGAGGAGGACCAGTGCATCTTTGAGC
ATCGCTACTTCAAGGCCAATGACACGCTGGGCTTCATGCTTATGTTGGCTGTGCTCATGGCAGCTACCCATGCTGTCTACGG
CAAGCTGCTCCTCTTCGAGTATCGTCACCGCAAGATGAAGCCAGTGCAGATGGTGCCAGCCATCAGCCAGAACTGGACATTC
CATGGTCCCGGGGCCACCGGCCAGGCTGCTGCCAACTGGATCGCCGGCTTTGGCCGTGGGCCCATGCCACCAACCCTGCTGG
GTATCCGGCAGAATGGGCATGCAGCCAGCCGGCGGCTACTGGGCATGGACGAGGTCAAGGGTGAAAAGCAGCTGGGCCGCAT
GTTCTACGCGATCACACTGCTCTTTCTGCTCCTCTGGTCACCCTACATCGTGGCCTGCTACTGGCGAGTGTTTGTGAAAGCC
TGTGCTGTGCCCCACCGCTACCTGGCCACTGCTGTTTGGATGAGCTTCGCCCAGGCTGCCGTCAACCCAATTGTCTGCTTCC
TGCTCAACAAGGACCTCAAGAAGTGCCTGAGGACTCACGCCCCCTGCGCGGCCGCACGGGGACGCACCCCACCCAGCCTGGG
TCCCCAAGATGAGTCCTGCACCACCGCCAGCTCCTCCCTGGCCAAGGACACTTCATCGTGA

SEQ ID No:66

Amino Acid sequence of the wild-type hSREB2 ACCESSION NP_061843

MANYSHAADNILQNLSPLTAFLKLTSLGFIIGVSVVGNLLISILLVKDKTLHRAPYYFLLDLCCSDILRSAICFPFVFNS
VKNGSTWTYGTLTCKVIAFLGVLSCFHTAFMLFCISVTRYLAIAHHRFYTKRLTFWTCLAVICMVWTLSVAMAFPPVLDV
GTYSFIREEDQCTFQHRSFRANDSLGFMLLLALILLATQLVYLKLIFFVHDRRKMKPVQFVAAVSQNWTFHGPGASGQAA
ANWLAGFGRGPTPPTLLGIRQNANTTGRRRLLVLDEFKMEKRISRMFYIMTFLFLTLWGPYLVACYWRVFARGPVVPGGF
LTAAVWMSFAQAGINPFVCIFSNRELRRCFSTTLLYCRKSRLPREPYCVI

SEQ ID No:67

Nucleotide sequence of the wild-type hSREB2 ACCESSION NM_018970

ATGGCGAACTATAGCCATGCAGCTGACAACATTTTGCAAAATCTCTCGCCTCTAACAGCCTTTCTGAAACTGACTTCCTT
GGGTTTCATAATAGGAGTCAGCGTGGTGGGCAACCTCCTGATCTCCATTTTGCTAGTGAAAGATAAGACCTTGCATAGAG
CACCTTACTACTTCCTGTTGGATCTTTGCTGTTCAGATATCCTCAGATCTGCAATTTGTTTCCCATTTGTGTTCAACTCT
GTCAAAAATGGCTCTACCTGGACTTATGGGACTCTGACTTGCAAAGTGATTGCCTTTCTGGGGGTTTTGTCCTGTTTCCA
CACTGCTTTCATGCTCTTCTGCATCAGTGTCACCAGATACTTAGCTATCGCCCATCACCGCTTCTATACAAAGAGGCTGA
CCTTTTGGACGTGTCTGGCTGTGATCTGTATGGTGTGGACTCTGTCTGTGGCCATGGCATTTCCCCCGGTTTTAGACGTG
GGCACTTACTCATTCATTAGGGAGGAAGATCAATGCACCTTCCAACACCGCTCCTTCAGGGCTAATGATTCCTTAGGATT
TATGCTGCTTCTTGCTCTCATCCTCCTAGCCACACAGCTTGTCTACCTCAAGCTGATATTTTTCGTCCACGATCGAAGAA
AAATGAAGCCAGTCCAGTTTGTAGCAGCAGTCAGCCAGAACTGGACTTTTCATGGTCCTGGAGCCAGTGGCCAGGCAGCT
GCCAATTGGCTAGCAGGATTTGGAAGGGGTCCCACACCACCCACCTTGCTGGGCATCAGGCAAAATGCAAACACCACAGG
CAGAAGAAGGCTATTGGTCTTAGACGAGTTCAAAATGGAGAAAAGAATCAGCAGAATGTTCTATATAATGACTTTTCTGT
TTCTAACCTTGTGGGGCCCCTACCTGGTGGCCTGTTATTGGAGAGTTTTTGCAAGAGGGCCTGTAGTACCAGGGGGATTT
CTAACAGCTGCTGTCTGGATGAGTTTTGCCCAAGCAGGAATCAATCCTTTTGTCTGCATTTTCTCAAACAGGGAGCTGAG
GCGCTGTTTCAGCACAACCCTTCTTTACTGCAGAAAATCCAGGTTACCAAGGGAACCTTACTGTGTTATATGA

SEQ ID No:68

Amino Acid sequence of the HA tagged wild-type hSREB2

MYPYDVPDYAAAAAMANYSHAADNILQNLSPLTAFLKLTSLGFIIGVSVVGNLLISILLVKDKTLHRAPYYFLLDLCCSDI
LRSAICFPFVFNSVKNGSTWTYGTLTCKVIAFLGVLSCFHTAFMLFCISVTRYLAIAHHRFYTKRLTFWTCLAVICMVWTL
SVAMAFPPVLDVGTYSFIREEDQCTFQHRSFRANDSLGFMLLLALILLATQLVYLKLIFFVHDRRKMKPVQFVAAVSQNWT
FHGPGASGQAAANWLAGFGRGPTPPTLLGIRQNANTTGRRRLLVLDEFKMEKRISRMFYIMTFLFLTLWGPYLVACYWRVF
ARGPVVPGGFLTAAVWMSFAQAGINPFVCIFSNRELRRCFSTTLLYCRKSRLPREPYCVI

SEQ ID No:69

Nucleotide sequence of the HA tagged wild-type hSREB2

ATGTACCCATACGACGTACCTGATTACGCAGCAGCAGCAGCAATGGCGAACTATAGCCATGCAGCTGACAACATTTTGCAA
AATCTCTCGCCTCTAACAGCCTTTCTGAAACTGACTTCCTTGGGTTTCATAATAGGAGTCAGCGTGGTGGGCAACCTCCTG
ATCTCCATTTTGCTAGTGAAAGATAAGACCTTGCATAGAGCACCTTACTACTTCCTGTTGGATCTTTGCTGTTCAGATATC
CTCAGATCTGCAATTTGTTTCCCATTTGTGTTCAACTCTGTCAAAAATGGCTCTACCTGGACTTATGGGACTCTGACTTGC
AAAGTGATTGCCTTTCTGGGGGTTTTGTCCTGTTTCCACACTGCTTTCATGCTCTTCTGCATCAGTGTCACCAGATACTTA
GCTATCGCCCATCACCGCTTCTATACAAAGAGGCTGACCTTTTGGACGTGTCTGGCTGTGATCTGTATGGTGTGGACTCTG
TCTGTGGCCATGGCATTTCCCCCGGTTTTAGACGTGGGCACTTACTCATTCATTAGGGAGGAAGATCAATGCACCTTCCAA
CACCGCTCCTTCAGGGCTAATGATTCCTTAGGATTTATGCTGCTTCTTGCTCTCATCCTCCTAGCCACACAGCTTGTCTAC
CTCAAGCTGATATTTTTCGTCCACGATCGAAGAAAAATGAAGCCAGTCCAGTTTGTAGCAGCAGTCAGCCAGAACTGGACT
TTTCATGGTCCTGGAGCCAGTGGCCAGGCAGCTGCCAATTGGCTAGCAGGATTTGGAAGGGGTCCCACACCACCCACCTTG
CTGGGCATCAGGCAAAATGCAAACACCACAGGCAGAAGAAGGCTATTGGTCTTAGACGAGTTCAAAATGGAGAAAAGAATC
AGCAGAATGTTCTATATAATGACTTTTCTGTTTCTAACCTTGTGGGGCCCCTACCTGGTGGCCTGTTATTGGAGAGTTTTT
GCAAGAGGGCCTGTAGTACCAGGGGGATTTCTAACAGCTGCTGTCTGGATGAGTTTTGCCCAAGCAGGAATCAATCCTTTT
GTCTGCATTTTCTCAAACAGGGAGCTGAGGCGCTGTTTCAGCACAACCCTTCTTTACTGCAGAAAATCCAGGTTACCAAGG
GAACCTTACTGTGTTATATGA

SEQ ID No:70

Amino Acid sequence of the hSREB2- Enhanced Receptor

MANYSHAADNILQNLSPLTAFLKLTSLGFIIGVSVVGNLLISILLVKDKTLHRAPYYFLLDLCCSDILRSAICFPFVFNS
VKNGSTWTYGTLTCKVIAFLGVLSCFHTAFMLFCISVTRYLAIAHHRFYTKRLTFWTCLAVICMVWTLSVAMAFPPVLDV
GTYSFIREEDQCTFQHRSFRANDSLGFMLLLALILLATQLVYLKLIFFVHDRRKMKPVQFVAAVSQNWTFHGPGASGQAA
ANWLAGFGRGPTPPTLLGIRQNANTTGRRRLLVLDEFKMEKRISRMFYIMTFLFLTLWGPYLVACYWRVFARGPVVPGGF
LTAAVWMSFAQAGINPFVCIFSNRELRRCFSTTLLYCAAARGRTPPSLGPQDESCTTASSSLAKDTSS

SEQ ID No:71

Nucleotide sequence of the hSREB2- Enhanced Receptor

ATGGCGAACTATAGCCATGCAGCTGACAACATTTTGCAAAATCTCTCGCCTCTAACAGCCTTTCTGAAACTGACTTCCTT
GGGTTTCATAATAGGAGTCAGCGTGGTGGGCAACCTCCTGATCTCCATTTTGCTAGTGAAAGATAAGACCTTGCATAGAG
CACCTTACTACTTCCTGTTGGATCTTTGCTGTTCAGATATCCTCAGATCTGCAATTTGTTTCCCATTTGTGTTCAACTCT
GTCAAAAATGGCTCTACCTGGACTTATGGGACTCTGACTTGCAAAGTGATTGCCTTTCTGGGGGTTTTGTCCTGTTTCCA
CACTGCTTTCATGCTCTTCTGCATCAGTGTCACCAGATACTTAGCTATCGCCCATCACCGCTTCTATACAAAGAGGCTGA
CCTTTTGGACGTGTCTGGCTGTGATCTGTATGGTGTGGACTCTGTCTGTGGCCATGGCATTTCCCCCGGTTTTAGACGTG
GGCACTTACTCATTCATTAGGGAGGAAGATCAATGCACCTTCCAACACCGCTCCTTCAGGGCTAATGATTCCTTAGGATT
TATGCTGCTTCTtGCTCTCATCCTCCTAGCCACACAGCTTGTCTACCTCAAGCTGATATTTTTCGTCCACGATCGAAGAA
AAATGAAGCCAGTCCAGTTTGTAGCAGCAGTCAGCCAGAACTGGACTTTTCATGGTCCTGGAGCCAGTGGCCAGGCAGCT
GCCAATTGGCTAGCAGGATTTGGAAGGGGTCCCACACCACCCACCTTGCTGGGCATCAGGCAAAATGCAAACACCACAGG
CAGAAGAAGGCTATTGGTCTTAGACGAGTTCAAAATGGAGAAAAGAATCAGCAGAATGTTCTATATAATGACTTTTCTGT
TTCTAACCTTGTGGGGCCCCTACCTGGTGGCCTGTTATTGGAGAGTTTTTGCAAGAGGGCCTGTAGTACCAGGGGGATTT
CTAACAGCTGCTGTCTGGATGAGTTTTGCCCAAGCAGGAATCAATCCTTTTGTCTGCATTTTCTCAAACAGGGAGCTGAG
GCGCTGTTTCAGCACAACCCTTCTTTACTGCGCGGCCGCACGGGGACGCACCCCACCCAGCCTGGGTCCCCAAGATGAGT
CCTGCACCACCGCCAGCTCCTCCCTGGCCAAGGACACTTCATCGTGA

SEQ ID No:72

Amino Acid sequence of the HA tagged hSREB2- Enhanced Receptor

MYPYDVPDYAAAAAMANYSHAADNILQNLSPLTAFLKLTSLGFIIGVSVVGNLLISILLVKDKTLHRAPYYFLLDLCCSD
ILRSAICFPFVFNSVKNGSTWTYGTLTCKVIAFLGVLSCFHTAFMLFCISVTRYLAIAHHRFYTKRLTFWTCLAVICMVW
TLSVAMAFPPVLDVGTYSFIREEDQCTFQHRSFRANDSLGFMLLLALILLATQLVYLKLIFFVHDRRKMKPVQFVAAVSQ
NWTFHGPGASGQAAANWLAGFGRGPTPPTLLGIRQNANTTGRRRLLVLDEFKMEKRISRMFYIMTFLFLTLWGPYLVACY
WRVFARGPVVPGGFLTAAVWMSFAQAGINPFVCIFSNRELRRCFSTTLLYCAAARGRTPPSLGPQDESCTTASSSLAKDT
SS

SEQ ID No:73

Nucleotide sequence of the HA tagged hSREB2- Enhanced Receptor

ATGTACCCATACGACGTACCTGATTACGCAGCAGCAGCAGCAATGGCGAACTATAGCCATGCAGCTGACAACATTTTGCA
AAATCTCTCGCCTCTAACAGCCTTTCTGAAACTGACTTCCTTGGGTTTCATAATAGGAGTCAGCGTGGTGGGCAACCTCC
TGATCTCCATTTTGCTAGTGAAAGATAAGACCTTGCATAGAGCACCTTACTACTTCCTGTTGGATCTTTGCTGTTCAGAT
ATCCTCAGATCTGCAATTTGTTTCCCATTTGTGTTCAACTCTGTCAAAAATGGCTCTACCTGGACTTATGGGACTCTGAC
TTGCAAAGTGATTGCCTTTCTGGGGGTTTTGTCCTGTTTCCACACTGCTTTCATGCTCTTCTGCATCAGTGTCACCAGAT
ACTTAGCTATCGCCCATCACCGCTTCTATACAAAGAGGCTGACCTTTTGGACGTGTCTGGCTGTGATCTGTATGGTGTGG
ACTCTGTCTGTGGCCATGGCATTTCCCCCGGTTTTAGACGTGGGCACTTACTCATTCATTAGGGAGGAAGATCAATGCAC
CTTCCAACACCGCTCCTTCAGGGCTAATGATTCCTTAGGATTTATGCTGCTTCTtGCTCTCATCCTCCTAGCCACACAGC
TTGTCTACCTCAAGCTGATATTTTTCGTCCACGATCGAAGAAAAATGAAGCCAGTCCAGTTTGTAGCAGCAGTCAGCCAG
AACTGGACTTTTCATGGTCCTGGAGCCAGTGGCCAGGCAGCTGCCAATTGGCTAGCAGGATTTGGAAGGGGTCCCACACC
ACCCACCTTGCTGGGCATCAGGCAAAATGCAAACACCACAGGCAGAAGAAGGCTATTGGTCTTAGACGAGTTCAAAATGG
AGAAAAGAATCAGCAGAATGTTCTATATAATGACTTTTCTGTTTCTAACCTTGTGGGGCCCCTACCTGGTGGCCTGTTAT
TGGAGAGTTTTTGCAAGAGGGCCTGTAGTACCAGGGGGATTTCTAACAGCTGCTGTCTGGATGAGTTTTGCCCAAGCAGG
AATCAATCCTTTTGTCTGCATTTTCTCAAACAGGGAGCTGAGGCGCTGTTTCAGCACAACCCTTCTTTACTGCGCGGCCG
CACGGGGACGCACCCCACCCAGCCTGGGTCCCCAAGATGAGTCCTGCACCACCGCCAGCTCCTCCCTGGCCAAGGACACT
TCATCGTGA

SEQ ID No:74

Amino Acid sequence of the wild-type hGPR8 ACCESSION NP_005277.1

MQAAGHPEPLDSRGSFSLPTMGANVSQDNGTGHNATFSEPLPFLYVLLPAVYSGICAVGLTGNTAVILVILRAPKMKTVT
NVFILNLAVADGLFTLVLPVNIAEHLLQYWPFGELLCKLVLAVDHYNIFSSIYFLAVMSVDRYLVVLATVRSRHMPWRTY
RGAKVASLCVWLGVTVLVLPFFSFAGVYSNELQVPSCGLSFPWPERVWFKASRVYTLVLGFVLPVCTICVLYTDLLRRLR
AVRLRSGAKALGKARRKVTVLVLVVLAVCLLCWTPFHLASVVALTTDLPQTPLVISMSYVITSLXYANSCLNPFLYAFLD
DNFRKNFRSILRC

SEQ ID No:75

Nucleotide sequence of the wild-type hGPR8 ACCESSION NM_ 005286

ATGCAGGCCGCTGGGCACCCAGAGCCCCTTGACAGCAGGGGCTCCTTCTCCCTCCCCACGATGGGTGCCAACGTCTCTCA
GGACAATGGCACTGGCCACAATGCCACCTTCTCCGAGCCACTGCCGTTCCTCTATGTGCTCCTGCCCGCCGTGTACTCCG
GGATCTGTGCTGTGGGGCTGACTGGCAACACGGCCGTCATCCTTGTAATCCTAAGGGCGCCCAAGATGAAGACGGTGACC
AACGTGTTCATCCTGAACCTGGCCGTCGCCGACGGGCTCTTCACGCTGGTACTGCCCGTCAACATCGCGGAGCACCTGCT
GCAGTACTGGCCCTTCGGGGAGCTGCTCTGCAAGCTGGTGCTGGCCGTCGACCACTACAACATCTTCTCCAGCATCTACT
TCCTAGCCGTGATGAGCGTGGACCGATACCTGGTGGTGCTGGCCACCGTGAGGTCCCGCCACATGCCCTGGCGCACCTAC
CGGGGGGCGAAGGTCGCCAGCCTGTGTGTCTGGCTGGGCGTCACGGTCCTGGTTCTGCCCTTCTTCTCTTTCGCTGGCGT
CTACAGCAACGAGCTGCAGGTCCCAAGCTGTGGGCTGAGCTTCCCGTGGCCCGAGCGGGTCTGGTTCAAGGCCAGCCGTG
TCTACACTTTGGTCCTGGGCTTCGTGCTGCCCGTGTGCACCATCTGTGTGCTCTACACAGACCTCCTGCGCAGGCTGCGG
GCCGTGCGGCTCCGCTCTGGAGCCAAGGCTCTAGGCAAGGCCAGGCGGAAGGTGACCGTCCTGGTCCTCGTCGTGCTGGC
CGTGTGCCTCCTCTGCTGGACGCCCTTCCACCTGGCCTCTGTCGTGGCCCTGACCACGGACCTGCCCCAGACCCCACTGG
TCATCAGTATGTCCTACGTCATCACCAGCCTCASSTACGCCAACTCGTGCCTGAACCCCTTCCTCTACGCCTTTCTAGAT
GACAACTTCCGGAAGAACTTCCGCAGCATATTGCGGTGCTGA

SEQ ID No:76

Amino Acid sequence of the HA tagged wild-type hGPR8

MYPYDVPDYAAAAAMQAAGHPEPLDSRGSFSLPTMGANVSQDNGTGHNATFSEPLPFLYVLLPAVYSGICAVGLTGNTAVI
LVILRAPKMKTVTNVFILNLAVADGLFTLVLPVNIAEHLLQYWPFGELLCKLVLAVDHYNIFSSIYFLAVMSVDRYLVVLA
TVRSRHMPWRTYRGAKVASLCVWLGVTVLVLPFFSFAGVYSNELQVPSCGLSFPWPERVWFKASRVYTLVLGFVLPVCTIC
VLYTDLLRRLRAVRLRSGAKALGKARRKVTVLVLVVLAVCLLCWTPFHLASVVALTTDLPQTPLVISMSYVITSLXYANSC
LNPFLYAFLDDNFRKNFRSILRC

SEQ ID No:77

Nucleotide sequence of the HA tagged wild-type hGPR8

ATGTACCCATACGACGTACCTGATTACGCAGCAGCAGCAGCAATGCAGGCCGCTGGGCACCCAGAGCCCCTTGACAGCAGG
GGCTCCTTCTCCCTCCCCACGATGGGTGCCAACGTCTCTCAGGACAATGGCACTGGCCACAATGCCACCTTCTCCGAGCCA
CTGCCGTTCCTCTATGTGCTCCTGCCCGCCGTGTACTCCGGGATCTGTGCTGTGGGGCTGACTGGCAACACGGCCGTCATC
CTTGTAATCCTAAGGGCGCCCAAGATGAAGACGGTGACCAACGTGTTCATCCTGAACCTGGCCGTCGCCGACGGGCTCTTC
ACGCTGGTACTGCCCGTCAACATCGCGGAGCACCTGCTGCAGTACTGGCCCTTCGGGGAGCTGCTCTGCAAGCTGGTGCTG
GCCGTCGACCACTACAACATCTTCTCCAGCATCTACTTCCTAGCCGTGATGAGCGTGGACCGATACCTGGTGGTGCTGGCC
ACCGTGAGGTCCCGCCACATGCCCTGGCGCACCTACCGGGGGGCGAAGGTCGCCAGCCTGTGTGTCTGGCTGGGCGTCACG
GTCCTGGTTCTGCCCTTCTTCTCTTTCGCTGGCGTCTACAGCAACGAGCTGCAGGTCCCAAGCTGTGGGCTGAGCTTCCCG
TGGCCCGAGCGGGTCTGGTTCAAGGCCAGCCGTGTCTACACTTTGGTCCTGGGCTTCGTGCTGCCCGTGTGCACCATCTGT
GTGCTCTACACAGACCTCCTGCGCAGGCTGCGGGCCGTGCGGCTCCGCTCTGGAGCCAAGGCTCTAGGCAAGGCCAGGCGG
AAGGTGACCGTCCTGGTCCTCGTCGTGCTGGCCGTGTGCCTCCTCTGCTGGACGCCCTTCCACCTGGCCTCTGTCGTGGCC
CTGACCACGGACCTGCCCCAGACCCCACTGGTCATCAGTATGTCCTACGTCATCACCAGCCTCASSTACGCCAACTCGTGC
CTGAACCCCTTCCTCTACGCCTTTCTAGATGACAACTTCCGGAAGAACTTCCGCAGCATATTGCGGTGCTGA

SEQ ID No:78

Amino Acid sequence of the hGPR8- Enhanced Receptor

MQAAGHPEPLDSRGSFSLPTMGANVSQDNGTGHNATFSEPLPFLYVLLPAVYSGICAVGLTGNTAVILVILRAPKMKTVT
NVFILNLAVADGLFTLVLPVNIAEHLLQYWPFGELLCKLVLAVDHYNIFSSIYFLAVMSVDRYLVVLATVRSRHMPWRTY
RGAKVASLCVWLGVTVLVLPFFSFAGVYSNELQVPSCGLSFPWPERVWFKASRVYTLVLGFVLPVCTICVLYTDLLRRLR
AVRLRSGAKALGKARRKVTVLVLVVLAVCLLCWTPFHLASVVALTTDLPQTPLVISMSYVITSLSYANSCLNPFLYAFLD
DNFRKNFRSILRCAAARGRTPPSLGPQDESCTTASSSLAKDTSS

SEQ ID No:79

Nucleotide sequence of the hGPR8- Enhanced Receptor

ATGCAGGCCGCTGGGCACCCAGAGCCCCTTGACAGCAGGGGCTCCTTCTCCCTCCCCACGATGGGTGCCAACGTCTCTCA
GGACAATGGCACTGGCCACAATGCCACCTTCTCCGAGCCACTGCCGTTCCTCTATGTGCTCCTGCCCGCCGTGTACTCCG
GGATCTGTGCTGTGGGGCTGACTGGCAACACGGCCGTCATCCTTGTAATCCTAAGGGCGCCCAAGATGAAGACGGTGACC
AACGTGTTCATCCTGAACCTGGCCGTCGCCGACGGGCTCTTCACGCTGGTACTGCCCGTCAACATCGCGGAGCACCTGCT
GCAGTACTGGCCCTTCGGGGAGCTGCTCTGCAAGCTGGTGCTGGCCGTCGACCACTACAACATCTTCTCCAGCATCTACT
TCCTAGCCGTGATGAGCGTGGACCGATACCTGGTGGTGCTGGCCACCGTGAGGTCCCGCCACATGCCCTGGCGCACCTAC
CGGGGGGCGAAGGTCGCCAGCCTGTGTGTCTGGCTGGGCGTCACGGTCCTGGTTCTGCCCTTCTTCTCTTTCGCTGGCGT
CTACAGCAACGAGCTGCAGGTCCCAAGCTGTGGGCTGAGCTTCCCGTGGCCCGAGCGGGTCTGGTTCAAGGCCAGCCGTG
TCTACACTTTGGTCCTGGGCTTCGTGCTGCCCGTGTGCACCATCTGTGTGCTCTACACAGACCTCCTGCGCAGGCTGCGG
GCCGTGCGGCTCCGCTCTGGAGCCAAGGCTCTAGGCAAGGCCAGGCGGAAGGTGACCGTCCTGGTCCTCGTCGTGCTGGC
CGTGTGCCTCCTCTGCTGGACGCCCTTCCACCTGGCCTCTGTCGTGGCCCTGACCACGGACCTGCCCCAGACCCCACTGG
TCATCAGTATGTCCTACGTCATCACCAGCCTCAGCTACGCCAACTCGTGCCTGAACCCCTTCCTCTACGCCTTTCTAGAT
GACAACTTCCGGAAGAACTTCCGCAGCATATTGCGGTGCGCGGCCGCACGGGGACGCACCCCACCCAGCCTGGGTCCCCA
AGATGAGTCCTGCACCACCGCCAGCTCCTCCCTGGCCAAGGACACTTCATCGTGA

SEQ ID No:80

Amino Acid sequence of the HA tagged hGPR8- Enhanced Receptor

MYPYDVPDYAAAAAMQAAGHPEPLDSRGSFSLPTMGANVSQDNGTGHNATFSEPLPFLYVLLPAVYSGICAVGLTGNTAVI
LVILRAPKMKTVTNVFILNLAVADGLFTLVLPVNIAEHLLQYWPFGELLCKLVLAVDHYNIFSSIYFLAVMSVDRYLVVLA
TVRSRHMPWRTYRGAKVASLCVWLGVTVLVLPFFSFAGVYSNELQVPSCGLSFPWPERVWFKASRVYTLVLGFVLPVCTIC
VLYTDLLRRLRAVRLRSGAKALGKARRKVTVLVLVVLAVCLLCWTPFHLASVVALTTDLPQTPLVISMSYVITSLSYANSC
LNPFLYAFLDDNFRKNFRSILRCAAARGRTPPSLGPQDESCTTASSSLAKDTSS

SEQ ID No:81

Nucleotide sequence of the HA tagged hGPR8- Enhanced Receptor

ATGTACCCATACGACGTACCTGATTACGCAGCAGCAGCAGCAATGCAGGCCGCTGGGCACCCAGAGCCCCTTGACAGCAGG
GGCTCCTTCTCCCTCCCCACGATGGGTGCCAACGTCTCTCAGGACAATGGCACTGGCCACAATGCCACCTTCTCCGAGCCA
CTGCCGTTCCTCTATGTGCTCCTGCCCGCCGTGTACTCCGGGATCTGTGCTGTGGGGCTGACTGGCAACACGGCCGTCATC
CTTGTAATCCTAAGGGCGCCCAAGATGAAGACGGTGACCAACGTGTTCATCCTGAACCTGGCCGTCGCCGACGGGCTCTTC
ACGCTGGTACTGCCCGTCAACATCGCGGAGCACCTGCTGCAGTACTGGCCCTTCGGGGAGCTGCTCTGCAAGCTGGTGCTG
GCCGTCGACCACTACAACATCTTCTCCAGCATCTACTTCCTAGCCGTGATGAGCGTGGACCGATACCTGGTGGTGCTGGCC
ACCGTGAGGTCCCGCCACATGCCCTGGCGCACCTACCGGGGGGCGAAGGTCGCCAGCCTGTGTGTCTGGCTGGGCGTCACG
GTCCTGGTTCTGCCCTTCTTCTCTTTCGCTGGCGTCTACAGCAACGAGCTGCAGGTCCCAAGCTGTGGGCTGAGCTTCCCG
TGGCCCGAGCGGGTCTGGTTCAAGGCCAGCCGTGTCTACACTTTGGTCCTGGGCTTCGTGCTGCCCGTGTGCACCATCTGT
GTGCTCTACACAGACCTCCTGCGCAGGCTGCGGGCCGTGCGGCTCCGCTCTGGAGCCAAGGCTCTAGGCAAGGCCAGGCGG
AAGGTGACCGTCCTGGTCCTCGTCGTGCTGGCCGTGTGCCTCCTCTGCTGGACGCCCTTCCACCTGGCCTCTGTCGTGGCC
CTGACCACGGACCTGCCCCAGACCCCACTGGTCATCAGTATGTCCTACGTCATCACCAGCCTCAGCTACGCCAACTCGTGC
CTGAACCCCTTCCTCTACGCCTTTCTAGATGACAACTTCCGGAAGAACTTCCGCAGCATATTGCGGTGCGCGGCCGCACGG
GGACGCACCCCACCCAGCCTGGGTCCCCAAGATGAGTCCTGCACCACCGCCAGCTCCTCCCTGGCCAAGGACACTTCATCG
TGA

SEQ ID No:82

Amino Acid sequence of the wild-type hGPR22 ACCESSION NP_005286.1

MCFSPILEINMQSESNITVRDDIDDINTNMYQPLSYPLSFQVSLTGFLMLEIVLGLGSNLTVLVLYCMKSNLINSVSNII
TMNLHVLDVIICVGCIPLTIVILLLSLESNTALICCFHEACVSFASVSTAINVFAITLDRYDISVKPANRILTMGRAVML
MISIWIFSFFSFLIPFIEVNFFSLQSGNTWENKTLLCVSTNEYYTELGMYYHLLVQIPIFFFTVVVMLITYTKILQALNI
RIGTRFSTGQKKKARKKKTISLTTQHEATDMSQSSGGRNVVFGVRTSVSVIIALRRAVKRHRERRERQKRVFRMSLLIIS
TFLLCWTPISVLNTTILCLGPSDLLVKLRLCFLVMAYGTTIFHPLLYAFTRQKFQKVLKSKMKKRVVSIVEADPLPNNAV
IHNSWIDPKRNKKITFEDSEIREKCLVPQVVTD

SEQ ID No:83

Nucleotide sequence of the wild-type hGPR22 ACCESSION NM_ 005295

ATGTGTTTTTCTCCCATTCTGGAAATCAACATGCAGTCTGAATCTAACATTACAGTGCGAGATGACATTGATGACATCAA
CACCAATATGTACCAACCACTATCATATCCGTTAAGCTTTCAAGTGTCTCTCACCGGATTTCTTATGTTAGAAATTGTGT
TGGGACTTGGCAGCAACCTCACTGTATTGGTACTTTACTGCATGAAATCCAACTTAATCAACTCTGTCAGTAACATTATT
ACAATGAATCTTCATGTACTTGATGTAATAATTTGTGTGGGATGTATTCCTCTAACTATAGTTATCCTTCTGCTTTCACT
GGAGAGTAACACTGCTCTCATTTGCTGTTTCCATGAGGCTTGTGTATCTTTTGCAAGTGTCTCAACAGCAATCAACGTTT
TTGCTATCACTTTGGACAGATATGACATCTCTGTAAAACCTGCAAACCGAATTCTGACAATGGGCAGAGCTGTAATGTTA
ATGATATCCATTTGGATTTTTTCTTTTTTCTCTTTCCTGATTCCTTTTATTGAGGTAAATTTTTTCAGTCTTCAAAGTGG
AAATACCTGGGAAAACAAGACACTTTTATGTGTCAGTACAAATGAATACTACACTGAACTGGGAATGTATTATCACCTGT
TAGTACAGATCCCAATATTCTTTTTCACTGTTGTAGTAATGTTAATCACATACACCAAAATACTTCAGGCTCTTAATATT
CGAATAGGCACAAGATTTTCAACAGGGCAGAAGAAGAAAGCAAGAAAGAAAAAGACAATTTCTCTAACCACACAACATGA
GGCTACAGACATGTCACAAAGCAGTGGTGGGAGAAATGTAGTCTTTGGTGTAAGAACTTCAGTTTCTGTAATAATTGCCC
TCCGGCGAGCTGTGAAACGACACCGTGAACGACGAGAAAGACAAAAGAGAGTCTTCAGGATGTCTTTATTGATTATTTCT
ACATTTCTTCTCTGCTGGACACCAATTTCTGTTTTAAATACCACCATTTTATGTTTAGGCCCAAGTGACCTTTTAGTAAA
ATTAAGATTGTGTTTTTTAGTCATGGCTTATGGAACAACTATATTTCACCCTCTATTATATGCATTCACTAGACAAAAAT
TTCAAAAGGTCTTGAAAAGTAAAATGAAAAAGCGAGTTGTTTCTATAGTAGAAGCTGATCCCCTGCCTAATAATGCTGTA
ATACACAACTCTTGGATAGATCCTAAAAGAAACAAAAAAATTACCTTTGAAGATAGTGAAATAAGAGAAAAATGTTTAGT
GCCTCAGGTTGTCACAGACTAG

SEQ ID No:84

Amino Acid sequence of the HA tagged wild-type hGPR22

MYPYDVPDYAAAAAMCFSPILEINMQSESNITVRDDIDDINTNMYQPLSYPLSFQVSLTGFLMLEIVLGLGSNLTVLVLY
CMKSNLINSVSNIITMNLHVLDVIICVGCIPLTIVILLLSLESNTALICCFHEACVSFASVSTAINVFAITLDRYDISVK
PANRILTMGRAVMLMISIWIFSFFSFLIPFIEVNFFSLQSGNTWENKTLLCVSTNEYYTELGMYYHLLVQIPIFFFTVVV
MLITYTKILQALNIRIGTRFSTGQKKKARKKKTISLTTQHEATDMSQSSGGRNVVFGVRTSVSVIIALRRAVKRHRERRE
RQKRVFRMSLLIISTFLLCWTPISVLNTTILCLGPSDLLVKLRLCFLVMAYGTTIFHPLLYAFTRQKFQKVLKSKMKKRV
VSIVEADPLPNNAVIHNSWIDPKRNKKITFEDSEIREKCLVPQVVTD

SEQ ID No:85

Nucleotide sequence of the HA tagged wild-type hGPR22

ATGTACCCATACGACGTACCTGATTACGCAGCAGCAGCAGCAATGTGTTTTTCTCCCATTCTGGAAATCAACATGCAGTC
TGAATCTAACATTACAGTGCGAGATGACATTGATGACATCAACACCAATATGTACCAACCACTATCATATCCGTTAAGCT
TTCAAGTGTCTCTCACCGGATTTCTTATGTTAGAAATTGTGTTGGGACTTGGCAGCAACCTCACTGTATTGGTACTTTAC
TGCATGAAATCCAACTTAATCAACTCTGTCAGTAACATTATTACAATGAATCTTCATGTACTTGATGTAATAATTTGTGT
GGGATGTATTCCTCTAACTATAGTTATCCTTCTGCTTTCACTGGAGAGTAACACTGCTCTCATTTGCTGTTTCCATGAGG
CTTGTGTATCTTTTGCAAGTGTCTCAACAGCAATCAACGTTTTTGCTATCACTTTGGACAGATATGACATCTCTGTAAAA
CCTGCAAACCGAATTCTGACAATGGGCAGAGCTGTAATGTTAATGATATCCATTTGGATTTTTTCTTTTTTCTCTTTCCT
GATTCCTTTTATTGAGGTAAATTTTTTCAGTCTTCAAAGTGGAAATACCTGGGAAAACAAGACACTTTTATGTGTCAGTA
CAAATGAATACTACACTGAACTGGGAATGTATTATCACCTGTTAGTACAGATCCCAATATTCTTTTTCACTGTTGTAGTA
ATGTTAATCACATACACCAAAATACTTCAGGCTCTTAATATTCGAATAGGCACAAGATTTTCAACAGGGCAGAAGAAGAA
AGCAAGAAAGAAAAAGACAATTTCTCTAACCACACAACATGAGGCTACAGACATGTCACAAAGCAGTGGTGGGAGAAATG
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AGACAAAAGAGAGTCTTCAGGATGTCTTTATTGATTATTTCTACATTTCTTCTCTGCTGGACACCAATTTCTGTTTTAAA
TACCACCATTTTATGTTTAGGCCCAAGTGACCTTTTAGTAAAATTAAGATTGTGTTTTTTAGTCATGGCTTATGGAACAA
CTATATTTCACCCTCTATTATATGCATTCACTAGACAAAAATTTCAAAAGGTCTTGAAAAGTAAAATGAAAAAGCGAGTT
GTTTCTATAGTAGAAGCTGATCCCCTGCCTAATAATGCTGTAATACACAACTCTTGGATAGATCCTAAAAGAAACAAAAA
AATTACCTTTGAAGATAGTGAAATAAGAGAAAAATGTTTAGTGCCTCAGGTTGTCACAGACTAG

SEQ ID No:86

Amino Acid sequence of the hGPR22-Enhanced Receptor

MCFSPILEINMQSESNITVRDDIDDINTNMYQPLSYPLSFQVSLTGFLMLEIVLGLGSNLTVLVLYCMKSNLINSVSNII
TMNLHVLDVIICVGCIPLTIVILLLSLESNTALICCFHEACVSFASVSTAINVFAITLDRYDISVKPANRILTMGRAVML
MISIWIFSFFSFLIPFIEVNFFSLQSGNTWENKTLLCVSTNEYYTELGMYYHLLVQIPIFFFTVVVMLITYTKILQALNI
RIGTRFSTGQKKKARKKKTISLTTQHEATDMSQSSGGRNVVFGVRTSVSVIIALRRAVKRHRERRERQKRVFRMSLLIIS
TFLLCWTPISVLNTTILCLGPSDLLVKLRLCFLVMAYGTTIFHPLLYAFTRQKFQKVLKSKMKKRVVCAAARGRTPPSLG
PQDESCTTASSSLAKDTSS

SEQ ID No:87

Nucleotide sequence of the hGPR22-Enhanced Receptor

ATGTGTTTTTCTCCcaTTCTGGAAATCAACATGCAGTCTGAATCTAACATTACAGTGCGAGATGACATTGATGACATCAA
CACCAATATGTACCAACCACTATCATATCCGTTAAGCTTTCAAGTGTCTCTCACCGGATTTCTTATGTTAGAAATTGTGT
TGGGACTTGGCAGCAACCTCACTGTATTGGTACTTTACTGCATGAAATCCAACTTAATCAACTCTGTCAGTAACATTATT
ACAATGAATCTTCATGTACTTGATGTAATAATTTGTGTGGGATGTATTCCTCTAACTATAGTTATCCTTCTGCTTTCACT
GGAGAGTAACACTGCTCTCATTTGCTGTTTCCATGAGGCTTGTGTATCTTTTGCAAGTGTCTCAACAGCAATCAACGTTT
TTGCTATCACTTTGGACAGATATGACATCTCTGTAAAACCTGCAAACCGAATTCTGACAATGGGCAGAGCTGTAATGTTA
ATGATATCCATTTGGATTTTTTCTTTTTTCTCTTTCCTGATTCCTTTTATTGAGGTAAATTTTTTCAGTCTTCAAAGTGG
AAATACCTGGGAAAACAAGACACTTTTATGTGTCAGTACAAATGAATACTACACTGAACTGGGAATGTATTATCACCTGT
TAGTACAGATCCCAATATTCTTTTTCACTGTTGTAGTAATGTTAATCACATACACCAAAATACTTCAGGCTCTTAATATT
CGAATAGGCACAAGATTTTCAACAGGGCAGAAGAAGAAAGCAAGAAAGAAAAAGACAATTTCTCTAACCACACAACATGA
GGCTACAGACATGTCACAAAGCAGTGGTGGGAGAAATGTAGTCTTTGGTGTAAGAACTTCAGTTTCTGTAATAATTGCCC
TCCGGCGAGCTGTGAAACGACACCGTGAACGACGAGAAAGACAAAAGAGAGTCTTCAGGATGTCTTTATTGATTATTTCT
ACATTTCTTCTCTGCTGGACACCAATTTCTGTTTTAAATACCACCATTTTATGTTTAGGCCCAAGTGACCTTTTAGTAAA
ATTAAGATTGTGTTTTTTAGTCATGGCTTATGGAACAACTATATTTCACCCTCTATTATATGCATTCACTAGACAAAAAT
TTCAAAAGGTCTTGAAAAGTAAAATGAAAAAGCGAGTTGTTTGTGCGGCCGCACGGGGACGCACCCCACCCAGCCTGGGT
CCCCAAGATGAGTCCTGCACCACCGCCAGCTCCTCCCTGGCCAAGGACACTTCATCGTGA

SEQ ID No:88

Amino Acid sequence of the HA tagged hGPR22- Enhanced Receptor

MYPYDVPDYAAAAAMCFSPILEINMQSESNITVRDDIDDINTNMYQPLSYPLSFQVSLTGFLMLEIVLGLGSNLTVLVLY
CMKSNLINSVSNIITMNLHVLDVIICVGCIPLTIVILLLSLESNTALICCFHEACVSFASVSTAINVFAITLDRYDISVK
PANRILTMGRAVMLMISIWIFSFFSFLIPFIEVNFFSLQSGNTWENKTLLCVSTNEYYTELGMYYHLLVQIPIFFFTVVV
MLITYTKILQALNIRIGTRFSTGQKKKARKKKTISLTTQHEATDMSQSSGGRNVVFGVRTSVSVIIALRRAVKRHRERRE
RQKRVFRMSLLIISTFLLCWTPISVLNTTILCLGPSDLLVKLRLCFLVMAYGTTIFHPLLYAFTRQKFQKVLKSKMKKRV
VCAAARGRTPPSLGPQDESCTTASSSLAKDTSS

SEQ ID No:89

Nucleotide sequence of the HA tagged hGPR22-Enhanced Receptor

ATGTACCCATACGACGTACCTGATTACGCAGCAGCAGCAGCAATGTGTTTTTCTCCcaTTCTGGAAATCAACATGCAGTCT
GAATCTAACATTACAGTGCGAGATGACATTGATGACATCAACACCAATATGTACCAACCACTATCATATCCGTTAAGCTTT
CAAGTGTCTCTCACCGGATTTCTTATGTTAGAAATTGTGTTGGGACTTGGCAGCAACCTCACTGTATTGGTACTTTACTGC
ATGAAATCCAACTTAATCAACTCTGTCAGTAACATTATTACAATGAATCTTCATGTACTTGATGTAATAATTTGTGTGGGA
TGTATTCCTCTAACTATAGTTATCCTTCTGCTTTCACTGGAGAGTAACACTGCTCTCATTTGCTGTTTCCATGAGGCTTGT
GTATCTTTTGCAAGTGTCTCAACAGCAATCAACGTTTTTGCTATCACTTTGGACAGATATGACATCTCTGTAAAACCTGCA
AACCGAATTCTGACAATGGGCAGAGCTGTAATGTTAATGATATCCATTTGGATTTTTTCTTTTTTCTCTTTCCTGATTCCT
TTTATTGAGGTAAATTTTTTCAGTCTTCAAAGTGGAAATACCTGGGAAAACAAGACACTTTTATGTGTCAGTACAAATGAA
TACTACACTGAACTGGGAATGTATTATCACCTGTTAGTACAGATCCCAATATTCTTTTTCACTGTTGTAGTAATGTTAATC
ACATACACCAAAATACTTCAGGCTCTTAATATTCGAATAGGCACAAGATTTTCAACAGGGCAGAAGAAGAAAGCAAGAAAG
AAAAAGACAATTTCTCTAACCACACAACATGAGGCTACAGACATGTCACAAAGCAGTGGTGGGAGAAATGTAGTCTTTGGT
GTAAGAACTTCAGTTTCTGTAATAATTGCCCTCCGGCGAGCTGTGAAACGACACCGTGAACGACGAGAAAGACAAAAGAGA
GTCTTCAGGATGTCTTTATTGATTATTTCTACATTTCTTCTCTGCTGGACACCAATTTCTGTTTTAAATACCACCATTTTA
TGTTTAGGCCCAAGTGACCTTTTAGTAAAATTAAGATTGTGTTTTTTAGTCATGGCTTATGGAACAACTATATTTCACCCT
CTATTATATGCATTCACTAGACAAAAATTTCAAAAGGTCTTGAAAAGTAAAATGAAAAAGCGAGTTGTTTGTGCGGCCGCA
CGGGGACGCACCCCACCCAGCCTGGGTCCCCAAGATGAGTCCTGCACCACCGCCAGCTCCTCCCTGGCCAAGGACACTTCA
TCGTGA

SEQ ID No:90

Agonist-Independent Translocation of Arrestin-GFP to GPCRs in the Presence of GRK2-C20

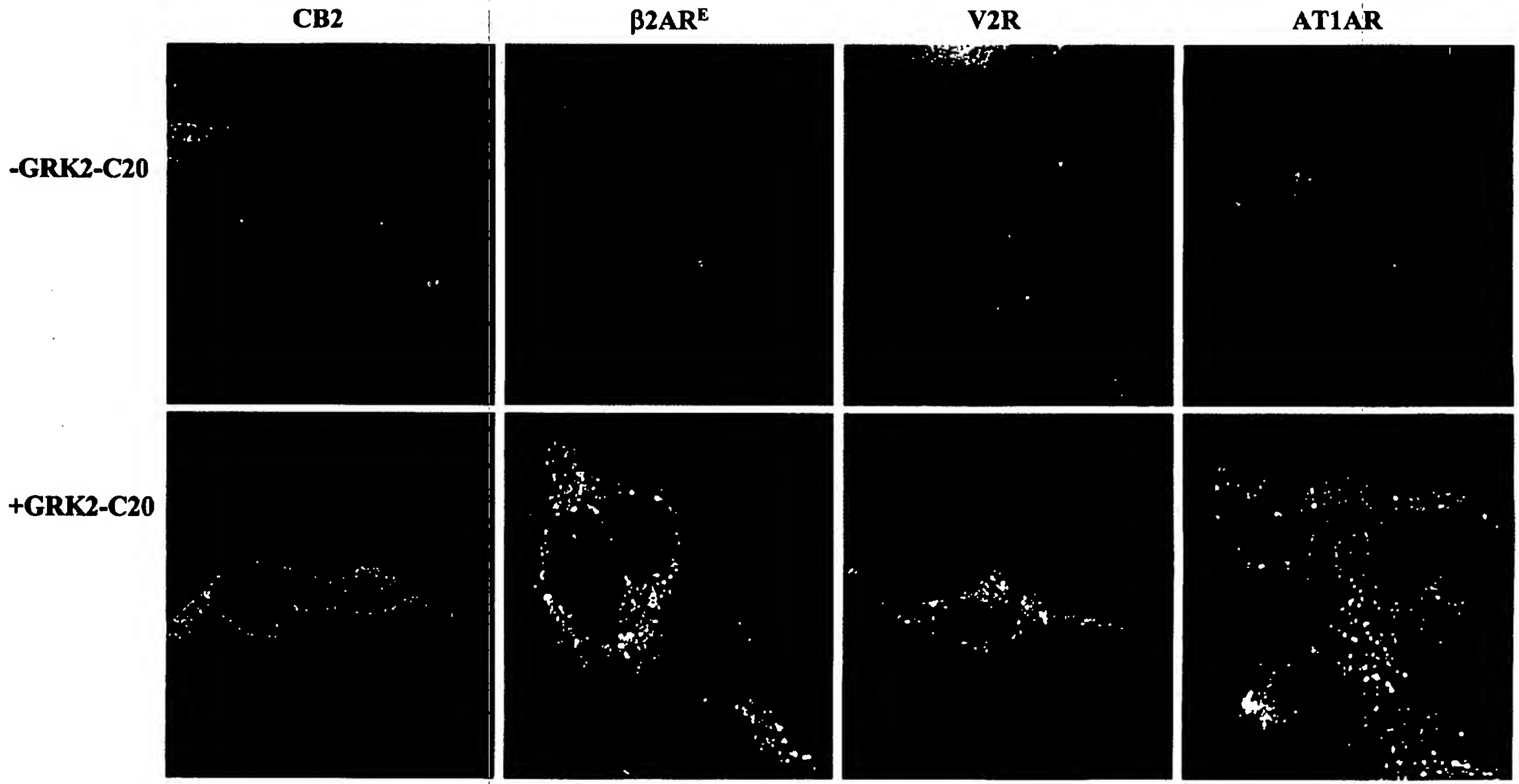

Fig. 4

Agonist-Independent Translocation of Arrestin-GFP to GPCRs in the Presence of GRK2-C20

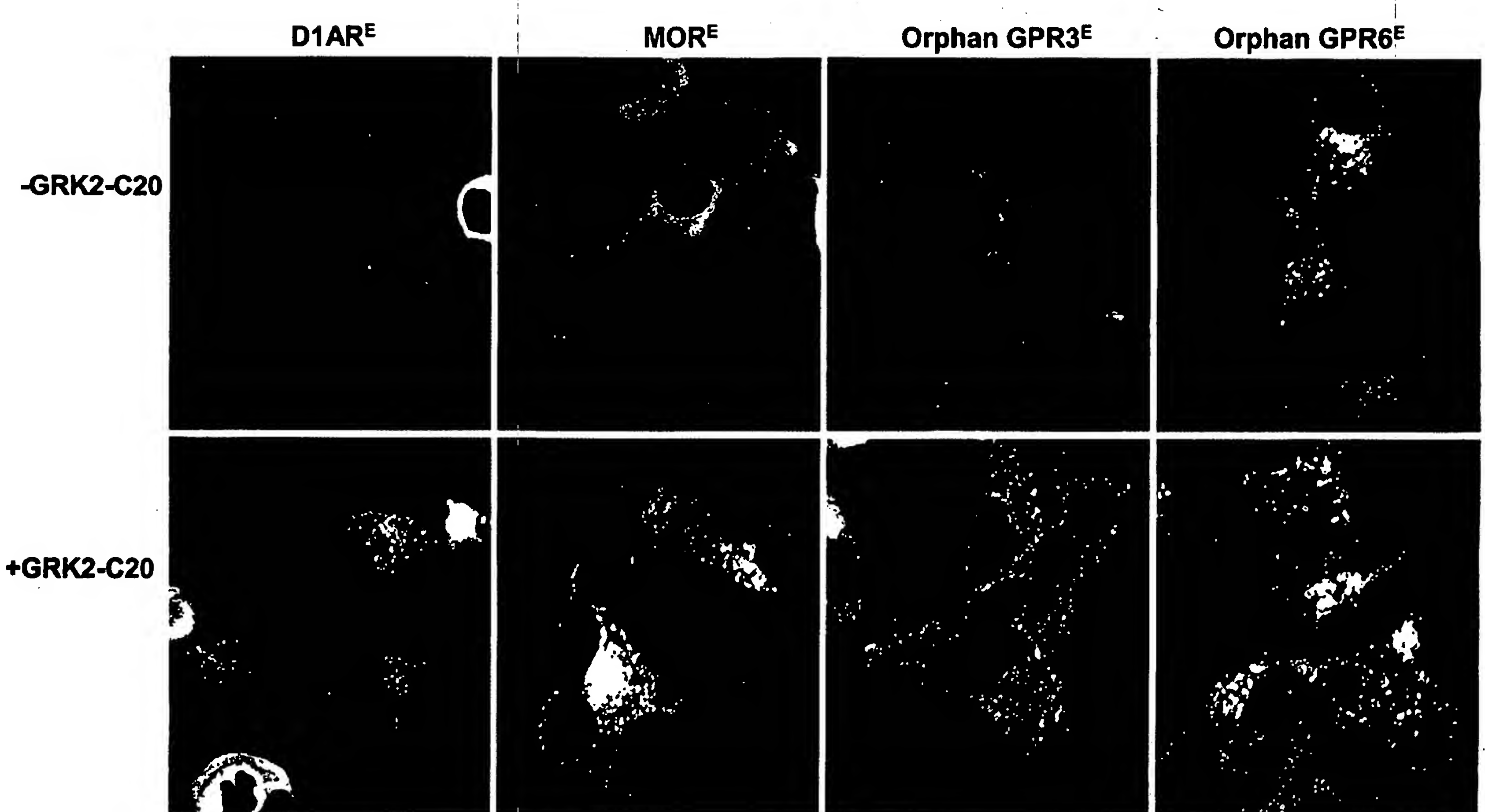

Fig. 5

Agonist-Independent Translocation of Arrestin-GFP to GPCRs in the Presence of GRK2-C20

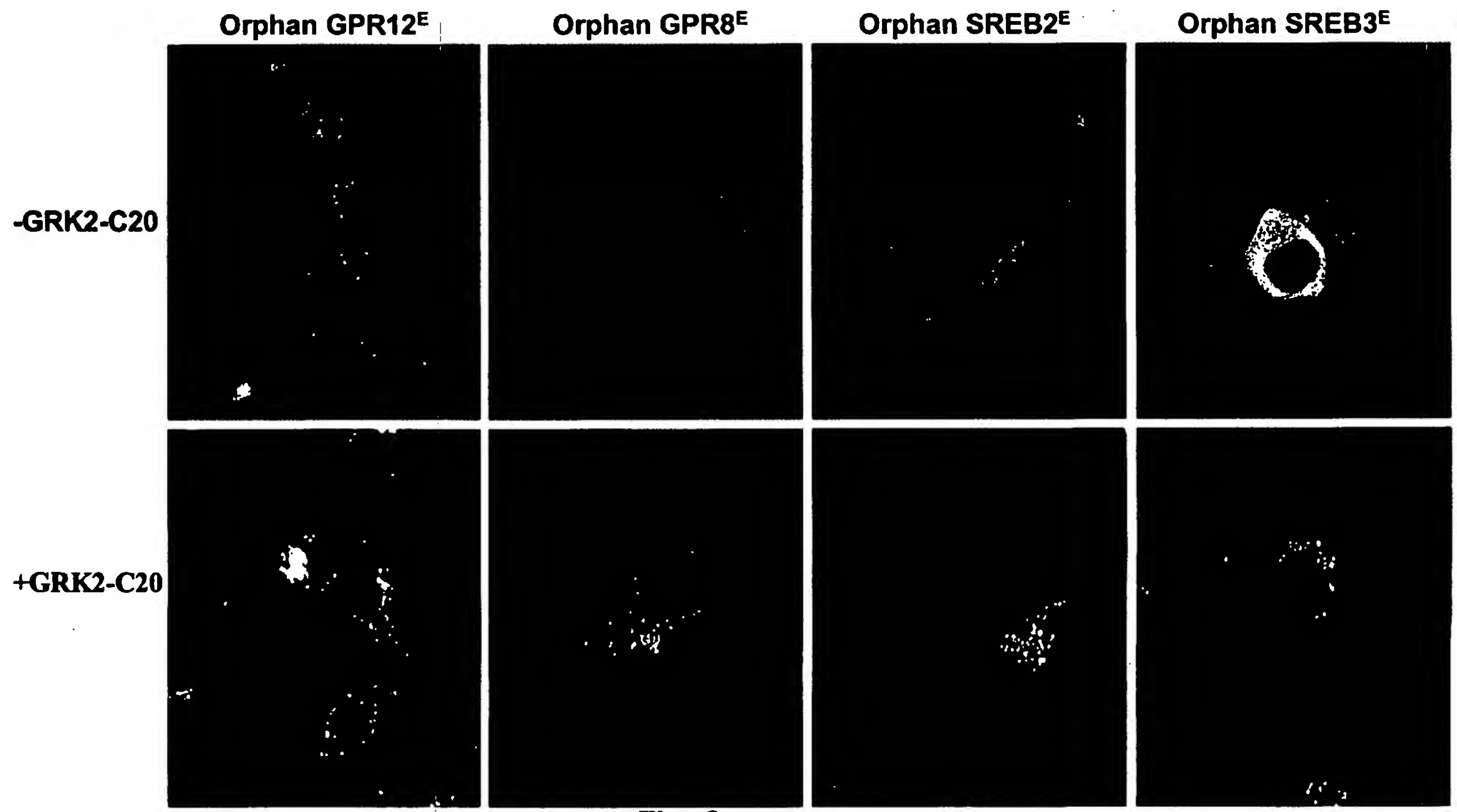

Fig. 6

Agonist-Independent Translocation of Arrestin-GFP to GPCRs in the Presence of GRK2-C20

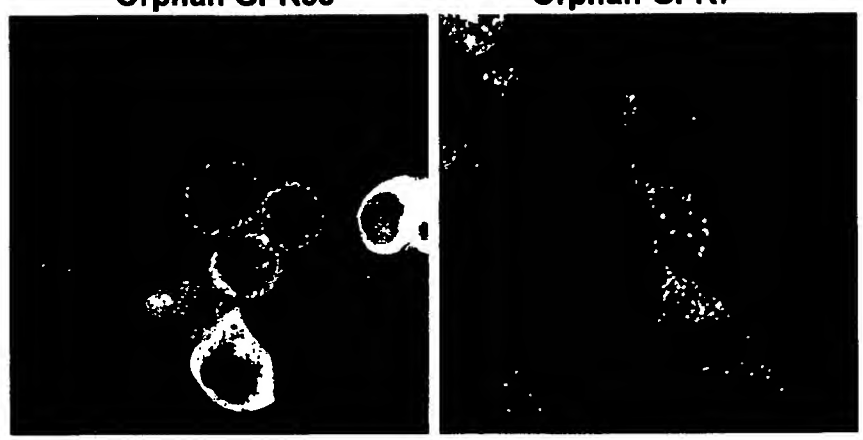

Fig. 7

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(71) Applicant *(for all designated States except US)*: NORAK BIOSCIENCES, INC. [US/US]; 7030 Kit Creek Rd., Morrisville, NC 27560 (US).

(72) Inventors; and
(75) Inventors/Applicants *(for US only)*: OAKLEY, Robert, H. [US/US]; 703 Braden Dr., Durham, NC 27713 (US). HUDSON, Christine, C. [US/US]; 201 Brandermill Dr., Durham, NC 27713 (US).

(74) Agents: BAUM, Allen, R. et al.; Burns, Doane, Swecker & Mathis, L.L.P., P.O. Box 1404, Alexandria, VA 27713 (US).

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(84) Designated States *(regional)*: ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IT, LU, MC, NL, PT, RO, SE, SI, SK, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Declarations under Rule 4.17:
— *as to applicant's entitlement to apply for and be granted a patent (Rule 4.17(ii)) for the following designations AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NI, NO, NZ, OM, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, UZ, VC, VN, YU, ZA, ZM, ZW, ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IT, LU, MC, NL, PT, RO, SE, SI, SK, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG)*
— *as to the applicant's entitlement to claim the priority of the earlier application (Rule 4.17(iii)) for all designations*
— *as to the applicant's entitlement to claim the priority of the earlier application (Rule 4.17(iii)) for all designations*

Published:
— *with international search report*
— *before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments*

(88) Date of publication of the international search report: 24 June 2004

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

WO 2003/097795 A3

(54) Title: CONSTITUTIVELY TRANSLOCATING CELL LINE

(57) Abstract: The present invention relates to agonist-independent methods of screening for compounds that alter GPCR desensitization. Included in the present invention are cell lines containing GRKs, in which GPCRs are desensitized in the absence of agonist; the GRKs may be modified. The present invention relates to methods to determine if a GPCR is expressed at the plasma membrane, and if the GPCR has an affinity for arrestin. Modified GPCRs which have increased arrestin affinity are included in the present invention. These modified GPCRs are useful in methods to screen for compounds that alter desensitization, including both the agonist- independent methods and agonist-dependent methods described herein.

INTERNATIONAL SEARCH REPORT	International application No. PCT/US03/14581

A. CLASSIFICATION OF SUBJECT MATTER

IPC(7) : C07K 14/705, 19/00; C12N 15/09, 15/62; G01N 33/53, 33/567
US CL : 435/7.2, 69.1, 69.7; 436/501; 530/350; 536/23.4, 23.5
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
U.S. : 435/7.2, 69.1, 69.7; 436/501; 530/350; 536/23.4, 23.5

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
Please See Continuation Sheet

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A --- X	US 5,891,646 (BARAK et al.) 06 April 1999 (06.04.1999), see entire document.	1-13 --- 14-27, 30-49
A --- X	GALES et al. Mutation of the Asn-391 within the Conserved NPXXY Motif of the Cholecystokinin B Receptor Abolishes Gq Protewin Activation without Affecting Its Association with theReceptor. The Journal of Biological Chemistry. 09 June 2000, Vol. 275, No. 23, pages 17321-17327, see abstract.	1-27 --- 37-49
A --- X	CHEN et al. Agonist-induced Internalization of the Platelet-activating Factor Receptor Is Dependant on Arrestins but Independant of G-protein Activation. The Journal of Biological Chemistry. 01March 2002, Vol. 277, No. 9, pages 7356-7362, see abstract.	1-27 --- 30-49
A --- X	INGLESE et al. Isoprenylation in regulation of signal transduction by G-protein-coupled receptor kinases. NATURE, 10 September 1992. Vol. 359, pages147-148, see entire document.	1-13 --- 30-36

☒ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search 17 March 2004 (17.03.2004)	Date of mailing of the international search report 04 MAY 2004
Name and mailing address of the ISA/US Mail Stop PCT, Attn: ISA/US Commissioner for Patents P.O. Box 1450 Alexandria, Virginia 22313-1450 Facsimile No. (703) 305-3230	Authorized officer Valerie Bell-Harris for John D. Ulm Telephone No. (571) 272-1600

PCT/US03/14581

INTERNATIONAL SEARCH REPORT

C. (Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A --- X	LOUDON et al. Altered Activity of Palmitoylation-deficient and Isoprenylated Forms of the G Protein-coupled Receptor Kinase GRK6. The Journal of Biological Chemistry, 24 October 1997, Vol. 272, No. 43, pages27422-27427, see abstract.	1-13 --------- 30-36

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US03/14581

Box I Observations where certain claims were found unsearchable (Continuation of Item 1 of first sheet)

This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claim Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☒ Claim Nos.: 28 and 29
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
Please See Continuation Sheet

3. ☐ Claim Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of Item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest ☐ The additional search fees were accompanied by the applicant's protest.
☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Continuation of Box I Reason 2:
Claim 28 is drawn to a compound that is defined only by a functional property detected in an assay, wherein it is not possible for one to determine if a given compound possesses this property without actually subjecting it to the claimed assay. Claim 29 is drawn to a method of treatment by administering a compound of claim 28.

Continuation of B. FIELDS SEARCHED Item 3:
U.S. Patents, STN/MEDLINE
search terms: agonist#, independant, receptor#, internalize?, kinase#, arrestin#, CAAX motinf, NPXXYmotif